EVALUATION OF A BOVINE TEMPERAMENT MODEL FOR ENDOPHENOTYPES ASSOCIATED WITH HYPOTHALAMIC-PITUITARY-ADRENAL AXIS DYSFUNCTION

A Dissertation

by

KEVIN OWEN CURLEY, JR.

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

May 2012

Major Subject: Physiology of Reproduction

Evaluation of a Bovine Temperament Model for Endophenotypes Associated with Hypothalamic-Pituitary-Adrenal Axis Dysfunction

EVALUATION OF A BOVINE TEMPERAMENT MODEL FOR ENDOPHENOTYPES ASSOCIATED WITH HYPOTHALAMIC-PITUITARY-ADRENAL AXIS DYSFUNCTION

A Dissertation

by

KEVIN OWEN CURLEY, JR.

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Approved by:

Co-Chairs of Committee,	Thomas H. Welsh, Jr.
	Ronald D. Randel
Committee Members,	Nancy H. Ing
	Douglas M. Stocco
	Jeffery A. Carroll
Head of Department,	H. Russell Cross

May 2012

Major Subject: Physiology of Reproduction

ABSTRACT

Evaluation of a Bovine Temperament Model for Endophenotypes Associated with Hypothalamic-Pituitary-Adrenal Axis Dysfunction.

(May 2012)

Kevin Owen Curley, Jr., B.S., University of Rhode Island; M.S., Texas A&M University

Co-Chairs of Advisory Committee: Dr. Thomas H. Welsh, Jr.
Dr. Ronald D. Randel

Dynamic interactions of behavior-related traits and the physiological stress response bear upon the beef industry by impacting animal welfare, health, and productivity. The specific mechanisms of hypothalamic-pituitary-adrenal (HPA) axis dysfunction as related to cattle temperament remain unclear. To further characterize endophenotypes associated with the complex interaction of environment and genotype, the following experiments focused on stimulation and regulation of the pituitary gland in cattle of differing genetic background and temperament.

Using serial blood sampling, via jugular cannula, the pituitary and subsequent adrenal response to exogenous vasopressin (VP) was characterized for steers of an excitable or calm temperament. Exit velocity (EV) measured at weaning was used to determine steer temperament. Endocrine parameters were measured for 6 h before and 6 h after the VP administration to quantify the stress response to both the handling associated with the experimental procedures and pharmacological challenge. Elevated concentrations of cortisol in excitable steers during the pre-challenge period reflected an

increased initial adrenal reactivity to interactions with humans. Subsequent acclimation to the experimental surroundings yielded greater baseline cortisol concentrations in the cattle with an excitable temperament. Pituitary stimulation with VP resulted in a greater adrenocorticotropic hormone (ACTH) output from the excitable compared to the calm animals.

A separate experiment employed the same 12-h blood sampling protocol with a different pituitary secretagogue, corticotrophin-releasing hormone (CRH), in order to evaluate pituitary-adrenal responsiveness in cattle with differing temperaments and genetic backgrounds. Measures of EV at weaning identified the calmest and most excitable steers from two separate calf crops; one Angus and the other Brahman. Within breed, adrenal medullary response to initial handling was influenced by temperament as concentrations of epinephrine and norepinephrine were higher in the excitable steers of both breedtypes. Additionally, concentrations of cortisol also differed by temperament in the Angus steers at this time point. An effect of temperament on pituitary responsiveness to exogenous CRH was observed in the Angus but not the Brahman steers. Unlike what was observed with the previously described VP challenge, the pituitary responsiveness to CRH was blunted in the excitable steers. The specific endophenotypes which have been identified or reinforced through these experiments suggest that there are aspects of HPA dysfunction associated with bovine temperament.

TABLE OF CONTENTS

CHAPTER Page

LIST OF FIGURES

Page

LIST OF TABLES

CHAPTER I

INTRODUCTION

As animal temperament is linked with stress physiology the benefits from decreasing numbers of temperamental animals within a herd may extend beyond behavior. It has been previously demonstrated that physiological stress responses associated with temperament persist throughout the course of a typical beef steer's lifetime. With stress responsiveness having biological links to growth performance, immunological proficiency, and meat quality there may be financial gains to come from reducing the number of temperamental cattle within a herd. Further elucidating the intricate relationship between cattle temperament and stress physiology may have broad impacts on animal welfare, health, and productivity within many facets of the beef industry. Currently, there is minimal understanding of the specific mechanisms that underpin hypothalamic-pituitary-adrenal (HPA) axis dysfunction as related to this behavioral paradigm. The characterization of specific endophenotypes inherent with cattle temperament would be helpful to ultimately unravel the complexities of this genotype-environment dynamic. Additionally, the usefulness of the bovine temperament model may extend beyond agriculture with implications for anxiety and stress-related pathologies in other species.

This dissertation follows the style of the Journal of Animal Science.

By using the temperament selection criterion of exit velocity, cattle typically exhibiting hypercortisolism and a blunted response to exogenous corticotrophin-releasing hormone (CRH) can be identified via individual behavioral responses to handling. To further characterize the HPA axis dysfunction associated with bovine temperament, this collection of experiments focused on the following:

1. the impact of temperament on pituitary and adrenal activity following stimulation with exogenous vasopressin (VP);

2. the effects of breedtype and temperament on pituitary and adrenal activity following stimulation with exogenous CRH; and

3. the use of a combined dexamethasone suppression and CRH challenge (DEX/CRH challenge) to identify altered endocrine feedback mechanisms linked to cattle temperament.

CHAPTER II

LITERATURE REVIEW

The Endocrine Stress Axis

Primer on Stress

Despite its relatively abstract nature and a history rife with confusion and controversy, the concept of stress is well recognized in the scientific, medical, and public communities. Though first proposed by Hans Selye (1936) and formally investigated for nearly three-quarters of a century, this concept is still best characterized only with a working definition: the biologic response by which an organism is coping with threats to homeostasis, (Moberg, 1999). An alarm reaction that consisted of at least; an enlargement of the adrenal glands, shrinkage of the thymus, spleen, and lymph nodes, and ulceration to the gastric mucosa (Selye, 1936), is the non-specific response that stress biology encompasses. Although this response has since been shown to be primarily mediated by the hypothalamus, anterior pituitary, and adrenal glands acting in concert, it can be elicited by a seemingly infinite number of events. However, the non-specificity of this response has also come under scrutiny (Pacak et al., 1998), since there appear to be finite differences in the specific components of the endocrine stress axis that are activated in response to different stressors.

The term “stressor” is used to qualify any such event that activates the hypothalamic-pituitary-adrenal (HPA) axis, regardless of the magnitude of response and which specific components are stimulated. Stressor-induced activation of the HPA axis

results in a cascade of endocrine mediated events that aid in coping with the particular stressful stimulus. Hypothalamic release of both corticotrophin-releasing hormone (CRH) and vasopressin (VP) stimulate corticotrophes of the anterior pituitary (Antoni, 1987; Aguilera, 1994; Grammatopoulos and Chrousos, 2002). Subsequent production of adrenocorticotropic hormone (ACTH) and release into peripheral circulation enables activation of its primary target tissue, the adrenal cortex (Axelrod and Reisine, 1984). Upon stimulation with ACTH, adrenal cortical tissue releases glucocorticoids (GC). The primary GC associated with stress responses are cortisol (human and domestic livestock) and corticosterone (rats, mice, birds and reptiles). To facilitate the coping response GCs elicit responses in a plethora of target tissues and alter many physiologic functions (Sapolsky et al., 2000). One important function is further regulation of the HPA through negative feedback mechanisms (Bradbury et al., 1991). Feedback upon both the hypothalamus and pituitary gland limit ACTH production to ultimately reduce further adrenal stimulation.

Unfortunately, the popular connotation of the word “stress” revolves around mental strain, anguish, or anxiety, thus much confusion can accompany explanations of stress biology. While stressors can be psycho-neural or psycho-social in nature, these are only a subset of possible stressors (Boissy, 1995). A proper discussion of stress should include any and all factors responsible for the activation of the HPA axis and the physiological consequences thereof.

The Adrenal Glands

Even during Selye's initial work with the alarm reaction, which was later designated the general adaptation syndrome (GAS; Selye, 1973), the adrenal glands were likely candidates for key involvement in the physiology behind this syndrome. Upon activation of the GAS, adrenal enlargement and the loss of both cortical lipoids and chromaffin substance were consistently observed (Selye, 1936). Ablation of the adrenals and subsequent attempts to activate the GAS did not result in typical thymic involution; however, adrenalectomy combined with injections of adrenal extracts did yield characteristic changes in the thymus (Selye, 1956). Thus, adrenal secretions were deemed necessary components of the GAS response. While it was demonstrated that multiple steroids could be crystallized from adrenal extracts, the preparation most effective in the bioassays utilized to test these purifications was water-soluble (Pfiffener, 1942; Mason, 1964). With the partial synthesis of cortisone, the steroid conformation of adrenal extracts, later named corticosteroids, was realized (Kendall, 1949).

The adrenal glands are composed of both steroid producing cortical tissue and catecholamine producing chromaffin tissue, also referred to as the adrenal medulla (Pohorecky and Wurtman, 1971). There is a compartmentalization of the adrenal cortex into three functionally-distinct zones; the outermost, mineralocorticoid synthesizing, zona glumerulosa (Kaplan and Bartter, 1962), the glucocorticoid producing zona fasciculata (Stachenko and Giroud, 1959), and the innermost, zona reticularis which synthesizes androgens (Cameron et al., 1969). While both the zona fasciculata and reticularis have the ability to produce a variety of steroids; the relative degree of

production of certain steroids would match their primary functions described above (Griffiths et al., 1963). Concerning the adrenals and GAS, glucocorticoids are the adrenal cortical steroids that are of primary importance as it is their replacement that maintains adrenalectomized animals in good health (Selye, 1971). During times of basal body maintenance, as well as in response to stressors, GCs have many functions. Such roles may encompass mediation of the immune response (Stenzel-Poore et al., 1993), anti-inflammation mechanisms (Hench et al., 1949), regulation of catecholamine synthesis (Pohorecky and Wurtman, 1971), and intervention of glucose homeostasis (Long et al., 1940). Glucocorticoids have been advocated as physiological indicators of the coping response to stressors since stress-induced activation of the HPA axis ultimately results in increased peripheral GC concentrations. As the early investigation of the stress response was being conducted, the complete picture of the HPA had not been fully realized and so the mechanisms by which the adrenals were regulated in times of stress were still an enigma.

The Role of the Pituitary Gland

The question of adrenal regulation became partially answered when investigation focused on the hypophysis, better known as the pituitary gland. During his early experiments with hypophysectomy, Smith (1930) observed significant atrophy of the adrenal cortex, but not the medulla. It was also demonstrated that pituitary extracts stimulated adrenal steroid production and release using an in vitro model (Hechter,

1949). At this time the pituitary extracts that elicited an adrenal response were commonly referred to as “cortin”, but were later identified as ACTH.

In addition to pituitary regulation of the adrenal glands, ideas of a negative feedback system where adrenal hormones inhibited pituitary stimulation of the adrenal glands began to develop. This was first demonstrated when administration of cortical extracts prevented adrenal cortex atrophy in response to large amounts of cortin (Ingle and Kendall, 1937). The actions of ACTH upon the adrenals were shown to occur quickly since the lag time between administration and adrenal cortical secretory actively was merely three minutes (Espiner et al., 1972). The molecular structure of ACTH was first proposed by Bell et al. (1956) but later revised and correctly identified (Riniker et al., 1972) as a single-chain polypeptide consisting of thirty-nine amino acid residues. Relative to the GAS, increased ACTH secretion from the adenohypophysis was observed following stimulation with noxious agents (Sydnor and Sayers, 1954) similar to those used in Selye’s initial work (1936). In the years following these early studies, the actions of ACTH on the adrenal glands during situations of stress have become widely accepted and well understood.

Hypothalamic Control of the Stress Response

The idea for hypothalamic mediation of the anterior pituitary gland was first put forth by Harris (1948) when he suggested that factors from the hypothalamus were transported via a portal blood network, from the median eminence, to elicit actions upon the adenohypophysis. Extracts from the median eminence were demonstrated to increase

ACTH secretion rate in median eminence lesioned rats (Royce and Sayers, 1960). The factor that regulates anterior pituitary release of ACTH, corticotropin-releasing hormone, has been identified as a peptide and was first sequenced to be a forty-one amino acid residue in ovids (Vale et al., 1981). Stimulation of the pars distalis, by CRH, enhances production of the polypeptide precursor molecule proopiomelanocortin (Childs, 1992) from which ACTH is cleaved (Mains et al., 1977). However, vasopressin, a peptide of hypothalamic origin, secreted from the posterior pituitary, has also been shown to induce ACTH secretion from the adenohypophysis (Martini and Morpurgo, 1955). Both CRH and VP have the ability to regulate functions of the corticotrophes separately but have also been demonstrated to act in synergy to stimulate ACTH release (Liu et al., 1983). Thus, it is the actions of both CRH and VP that constitute the hypothalamic contributions to the HPA axis.

Regulation via Feedback Mechanisms

Corticotrophs of the anterior pituitary are regulated by an amalgam of stimulatory and inhibitory inputs originating from the hypothalamus and adrenal gland. By binding to specific receptors for both CRH (Vale et al., 1981) and VP, these hormones individually and synergistically (Gillies et al., 1982) stimulate the production and release of pituitary ACTH into the systemic circulation. Alternatively, GCs serve as a negative feedback mechanism for the HPA axis via inhibitory actions at the pituitary (Keller-Wood and Dallman, 1984) as well as in the hypothalamus and higher brain centers (McEwen et al., 1986).

Feedback mechanisms of GCs on the HPA are mediated through distinct receptor subtypes; mineralocorticoid (MR) and glucocorticoid (GR) receptors (for review, see de Kloet et al., 1998). The MR is typified by a high affinity (Reul and de Kloet, 1985) for endogenous GC and believed to play a role in the regulation of basal HPA function (Arriza et al., 1988). Conversely, the GR are thought to be more important during a stress response (Young et al., 2004) and are typified by lower affinity for endogenous GC then MR, yet have a high affinity for dexamethasone and are the predominant receptor-type found in the pituitary (de Kloet et al. 1975). Co-localized populations of MR and GR have been observed in neurons of the hypothalamic paraventricular nucleus, as well as various structures within the limbic system, in a wide array of species (Patel et al. 2000), thus providing the mechanisms for feedback regulation atop the HPA axis as well as in higher brain centers.

The physiological ramifications of this dual-receptor system presents a complex system that will certainly not be easy to unravel, as evident by the over 200 GC-responsive genes that have been identified in the hippocampus alone (Datson et al. 2001). Moreover, in addition to receptor homodimerization, the ability for MR and GR to form functional monomers and heterodimers (Trapp et al., 1994; Nishi et al., 2001) allows for responsivity across a gamut of GC concentrations; during basal or stressed scenarios. This permits the relative degree of combined positive and negative input to finely regulate the endocrine HPA function both during basal and stressed states.

Cattle Temperament

Animal Temperament

As man began the domestication of animals, it was evident that individual behavioral responses to man differed greatly from species to species and also within herds or groups. Scott and Fredericson (1951) identified "tameness" and "wildness" as pertained to animal reactions towards man. They defined the term "tameness" as the absence of conflict behavior, and the term "wildness" as the tendency to escape. Notation of reactions of specific animals towards humans was valuable to handlers as it allowed for the identification and possible separation of those individuals that were easier to work with from those that needed precautions to be taken while handling them. Along with the various terms listed above, "temperament" has also been used to characterize behavior responses to human-animal interactions (Burrow, 1997). However, as Stricklin and Kautzscanavy (1984) point out, within the scientific community much misunderstanding has accompanied this term as researchers have used temperament when referring to the nervousness, skittishness, quietness, excitability, individuality, libido, constitution, and emotionality of animals. Boissy (1995) defines fear and anxiety as emotional states induced by the perception of actual danger (fear state) or potential danger (anxiety state). Assuming that a fear response underpins animal reactions toward man, then a case could be made for those animals of excitable temperament (i.e. those with a greater adverse response to human-handling) exhibiting a greater fear response in general. For the purposes of this discussion temperament will refer to the relative ease of eliciting such adverse reactions in an individual animal.

Temperament and Beef Production

The impact of animal temperament on livestock production has been investigated for more than fifty years. The relationship of nervousness to decreased conception rates (Pounden and Firebaugh, 1956) and greater energy requirements (Hafez and Lindsay, 1965) were among some early findings linking cattle temperament and economically important aspects of beef production. In relatively more recent studies, cattle with an excitable temperament exhibited decreased average daily gains when compared with calmer herd mates (Voisinet et al., 1997b; Petherick et al., 2002; Hoppe et al., 2010). These differences in gains translated into lower yearling body weights of progeny from sires with an excitable temperament (Burrow and Dillon, 1997). Moreover, lower body condition scores have also been related to an excitable temperament (Petherick et al., 2003). And finally, calmer temperament has been demonstrated to positively impact multiple measures of feedlot performance (Behrends et al., 2009).

The effects of temperament on performance in the feedlot may be directly related to overall animal health. Elevated rectal temperatures have been observed in bulls of an excitable temperament in response to transportation stress (Burdick et al., 2010). Animal handling associated with transport to the feedlot resulted in reduced neutrophil function and possible hindrance of the innate immune system in temperamental cattle (Hulbert et al., 2011). Furthermore, with the use of an endotoxin challenge Burdick and colleagues (2011b) observed a disparity in resultant sickness behavior across cattle temperaments. The interplay between temperament and stressors associated with arrival

to the feedlot has also been suggested to impact aspects of the humoral immune response (Fell et al., 1999). Taken together, these findings suggest that cattle temperament may be an important dynamic on animal health in the feedlot environment, and should therefore be considered as a potential means to lost productivity.

In addition to producing less commercial product, an excitable temperament affects meat quality. Meat tenderness is negatively impacted by temperament as Warner-Bratzler shear force measures were greater in steers with an excitable temperament when compared to calmer cattle (Voisinet et al., 1997a; King et al., 2006; Hall et al., 2011). Total yield may also be influenced by temperament, as carcasses from excitable cattle yield less meat due to increased amounts of bruise trim from injuries acquired during transportation (Fordyce et al., 1988b). Also, increased percentages of borderline dark cutters have been observed in meat from cattle with an excitable temperament when compared to that from calm cattle (Voisinet et al., 1997a).

The summation of all these findings demonstrates that temperament may have a bearing on many facets of beef production and that, in general, an excitable temperament is undesirable in the beef industry.

Temperament Assessment

Various methodologies have been devised and implicated in the assessment of cattle temperament for scientific inquiry. Many temperament assessment methodologies revolve around cattle behavior while confined to the chute, as these are commonly used during basic management practices within the cattle industry. Based on animal reaction

to entering the chute or weigh box and subsequent restraint with a head gate, a scoring technique was developed (Tulloh, 1961). Using this scoring technique, animal temperament was determined to be between 1 (docile) and 6 (aggressive). An example of a specific description for assigning a particular temperament score with the Tulloh method would be; "3, restless: an animal which moves almost continuously, pulling or pushing on sides of crush; stance is difficult to make observations on; flicks tail frequently, snorts; animal objects to having ear tag handled during identification; may be stubborn." Other very similar iterations of this method have been utilized in subsequent research (Dickson et al., 1970; Fordyce et al., 1988b; Grandin, 1993). In addition, the heritability of temperament assessed with such methodologies has been shown to be moderately to highly heritable (O'Bleness et al., 1960). Similar scoring methodologies have also been used while the animals were in an enclosed pen rather than confined within the chute (Hammond et al., 1996). Inherent to all the scoring methods of cattle temperament is the overtly subjective nature of these assessments, as variations between individuals scoring the animals can lead to quite different appraisals of cattle temperament.

Other non-subjective methodologies have also been utilized to quantify cattle temperament. Some commonly used methods regard the proximity to an animal that a human could maintain (Purcell et al., 1988). In the flight distance test, the shortest distance a human could come to a stationary animal before it moved away was determined; while, in the approach test the shortest distance to a stationary human that an animal would come was measured. However, these proximity measures were often

difficult to obtain, extremely time-consuming to implement under research conditions, and exponentially troublesome when incorporated into routine management practices (Burrow et al., 1988).

Measuring Temperament via Exit Velocity

Exit velocity (EV) provides an objective measure of cattle temperament that is relatively easy to implement under both management and research conditions. Burrow et al. (1988) demonstrated that the speed at which cattle exit the working chute was correlated to animal temperament. The cattle that exited the chute with faster velocities were of a more excitable temperament when compared to those that had a slower flight speed and were calmer. Additionally, the measures of flight speed have been shown to be correlated with measures of flight distance (Burrow, 1997). This measurement of EV specifically quantifies the relative degree of fear response generated by human handling of cattle (Burrow and Dillon, 1997) and therefore can be useful to explore the impact of this responsiveness during typical management scenarios. A fact that is demonstrated by the continued use of EV—or similar techniques identified as flight time or flight speed—to assess cattle temperament in a research setting (Fell et al., 1999; Petherick et al., 2002; Petherick et al., 2003; Burrow and Prayaga, 2004; King et al. 2006; Curley et al., 2008; Burdick et al., 2011b).

Reliability and Heritability of Exit Velocity

It is particularly important to note that this EV measure indicates the relative ease with which an individual is agitated by handling, with relative being the keyword in that statement. Much individual variation within and across various measures of temperament has been demonstrated in cattle (Kilgour et al., 2006). Concerning EV measures specifically, there have been conflicting reports on how these rates change over time. Exit velocities assessed very early in a calf's life (about 21 days of age) have been shown to increase as the animals approach weaning (Burdick et al., 2011a). Beginning at 8 mo. of age, three consecutive monthly-measurements resulted in a small increase in EV in heifers (Müller and von Keyserlingk, 2006). Additionally, Petherick and colleagues (2002) observed an increase in EV during the 100 d following arrival to the feedlot in repeated measurements of 2-year-old steers. Yet, yearling bulls identified to be the most excitable in the herd demonstrated slower EV when measured 4 mo. later (Curley et al., 2006). Moreover, in an experiment specifically focused on the impact of either positive or negative handling experiences on cattle temperament, repeated handling resulted in reduced exit velocity measures irrespective of the quality of handling (Petherick et al., 2009a).

Although there are discrepancies in observations of EV measures over time, general temperament appraisals via EV remain consistent. That is, when used to categorize cattle as having either an excitable or calm temperament these assessments, relative to those of herd mates, persist over time regardless of how EV values may vary

over repeated measurements (Curley et al., 2006; Petherick et al., 2009a; Burdick et al., 2011a).

Across a variety of assessment measures, temperament has been demonstrated to be a moderate to highly heritable (O'Bleness et al., 1960; Morris et al., 1994; Hoppe et al., 2010), independent trait (Fordyce et al., 1996; Burrow, 2001; Burrow and Prayaga, 2004). Heritability estimates for temperament specifically assessed with EV echo this sentiment. A composite of multiple EV measures, while in the feedlot, produced a heritability estimate of 0.34 in *Bos taurus* cattle (Rolfe et al., 2011). This was more conservative than the 0.49 previously observed with EV measured on cattle of a similar breed type and during the same phase of the beef production scheme (Nkrumah et al., 2007). Although, the relatively small number of animals (n = 302) used by Nkrumah and colleagues may render their estimate less reliable. Exit velocity recorded during times when the animals are in a less intensive management-environment yielded high heritability estimates that ranged between 0.4 and 0.44 (Burrow, 2001). While a single EV measurement would be sufficient, using multiple measures, at and after weaning, has been suggested for an increased selection response (Burrow and Corbet, 2000).

Exit Velocity and the HPA Axis

The endocrine stress axis would seem to be a likely candidate to explain the physiologic mechanisms by which temperament could negatively impact beef production, as described above. There is evidence of a direct relationship between adrenal responsiveness and temperament assessed via exit velocity. Fell and colleagues

(1999) first associated elevated concentrations of cortisol with faster EV measures. The differentiation of cortisol concentrations in groupings based on EV was shown to persist over multiple observations in bulls (Curley et al., 2006). The comparison of cortisol concentrations obtained from serial blood sampling has demonstrated increased adrenal activity in heifers with an excitable temperament, which remained distinguishable from calmer animals over a period of 6 hours (Curley et al., 2008). And like the adrenal cortex, there seems to be temperament-related effects on medullary function. Epinephrine concentrations were markedly higher in bulls of poor temperament as compared to their calmer counterparts (Burdick et al., 2010).

While experiments using exogenous challenge to the HPA axis have incorporated the temperament paradigm, their resultant data has yet to provide a full realization of the specific mechanisms by which cattle temperament impacts HPA function. Adrenal response to exogenous ACTH does not result in peak cortisol concentrations that differ with temperament (Curley et al., 2008; Cafe et al., 2011b). Therefore, it would seem that temperament may not impact the capacity for the adrenal glands to produce GCs. Stimulation of the pituitary with exogenous CRH also results in similar peak production of ACTH across temperament groups (Curley et al. 2008). Yet, this study did demonstrate an overall blunted response in the more excitable animals, at both the level of the pituitary and adrenal gland, when considering post-challenge reactivity over multiple hours. Furthermore, in reflecting on their data, the authors of both these studies were uncertain of the full extent in which the animal handling associated with the experimental procedures impacted the resolution of their data analysis. It is evident that

further inquiry into the dynamics of HPA function as related to cattle temperament could be useful to pursue.

Other Behavioral-related Models of HPA Dysfunction

Roman High and Low Avoidance Rats

A multitude of different behavioral-based murine models have been incorporated to investigate aberrations in the endocrine stress axis. Although, the specific behavioral trait measured could have been characterized as temperament, personality, excitability, or emotional reactivity. Once such model utilizes the Roman High (RHA) and Low (RLA) Avoidance rats, which are divergent lines based on selection for high and low conditioned avoidance responses (Bignami, 1965). Typically a two-way shuttle box test is used to gauge rapid versus non-acquisition of active avoidance behavior. Over forty years of research have characterized RLA rats as less active under unstimulated conditions, exhibiting increased emotional response to novel environments, and possessing a hyper-responsive HPA axis (for review, see Steimer and Driscoll, 2005).

Specifically, Gentsch et al. (1982) demonstrated increased corticosterone and ACTH responses to novel environments and psychosocial stressors in RLA compared to RHA rats. Additionally, RLA rats have been shown to exhibit an increased ACTH response to CRH challenge (Walker et al. 1989). Western blot analysis has demonstrated a 2:1 ratio (RLA:RHA rats) for CRH receptor expression in the pituitary (Steimer et al. 2007). Also, when comparing GR populations across RLA and RHA rat pituitaries, a decrease in receptor population was observed in the rats with increased

emotional reactivity (Walker et al. 1989). Currently the RLA/RHA model is used in multiple laboratories around the world as a tool for investigating the links of personality to health and disease.

High and Low Anxiety-Related Behavior Rats

High (HAB) and Low (LAB) Anxiety-related Behavior rats represent another widely used model for the link between gene-environment interactions and psychopathology (for review, see Landgraf and Wigger, 2002). Duration of time spent in the open arms of an elevated plus maze is the selection basis for these distinct lines, wherein HAB rats spend significantly less time, if they enter the open arms at all. The elevated plus maze is a useful tool for identifying animals with a predisposition to be anxious thus generating further insight into the mechanisms involved in mediating anxiety (Hogg, 1996).

Regarding HPA function, HAB rats respond to psychosocial stressors with increased pituitary and adrenal hormone production compared to LAB rats (Landgraf et al., 1999). Salomé et al. (2006) has supported this with the observations of increased pituitary POMC mRNA expression in HAB, following a mild stressor. But, unlike the RLA/RHA model, feedback regulation of corticotroph function may not be an explanation here, as Keck et al. (2002) did not find differing pituitary GR populations when comparing tissues from HAB and LAB rats. Ongoing research with the HAB/LAB model may prove to further clarify the mechanisms by which elevated HPA function is observed in anxious individuals.

High and Low Stress-Responsive Japanese Quail

It is important to note evidence of a reciprocal nature of a hyper-responsive HPA axis and fearfulness. Selection for increased corticosterone secretion, induced by immobilization, has yielded high (HS) and low (LS) stress responsive Japanese quail, respectively (Satterlee and Johnson, 1988). Behavioral tests comparing these lines have demonstrated an elevated fearfulness of humans associated with the HS quail (Jones et al., 1994).

Conclusions

The Rationale for Detailing Temperament-related Endophenotypes

As mood and anxiety disorders are likely resultant from multiple gene-environment interactions that evoke a variety of symptoms, the endophenotype approach serves to identify individual components of these psychopathologies. Characterization of these "internal" phenotypes (Gottesman and Shields, 1973) helps to construct a composite of the measurable biological markers associated with such complex disorders. In some cases a disease's pathophysiology may be straightforward (e.g. resultant from a single gene mutation); however, subtle genetic polymorphisms, exposure during critical periods, the impact of environmental events, and epigenetic modification serve to muddle the pathophysiology of other diseases (Hasler et al., 2004). Defining an endophenotypic profile for these more multifaceted pathologies could lead to the development of applicable animal partial-models necessary for greater understanding of

these disorders (Gottesman and Gould, 2003). In accordance with this, the bovine temperament model may be of use to elucidate physiological phenotypes or pathologies that have parallels in other species.

An endophenotype must exhibit a variety of important criteria in order to be useful in the study of psychopathologies. Such criteria include state-independence, heritability, and biological plausibility (Tsuang et al., 1993). Those described herein, as well as countless other studies, using a variety of methodologies to characterize emotional reactivity, have forged a strong hypothesis linking fearfulness, psychological disorders, and altered HPA function. It is well within the realm of biological plausibility that the HPA dysfunction previously observed, e.g., hypercortisolism (Curley et al., 2006) and blunted response to CRH challenge (Curley et al., 2008), as well as others yet to be determined, are relevant characteristics of the bovine temperament model. Since temperament assessments have been demonstrated to be reliable over time (Curley et al., 2006; Petherick et al., 2009a; Burdick et al., 2011a), our model would fit the state-independence criterion also. Gould and Gottesman (2006) articulated this specific criterion further by stipulating that sometimes the endophenotype may require challenge or provocation to manifest; lending further credence to hypercortisolism associated with an excitable temperament being a relevant characteristic. As temperament has been demonstrated to be at least moderately heritable (Burrow, 2001; Rolfe et al., 2011), our model meets another of Tsuang's (1993) criterion. Moreover, Bearden and Freimer (2006) explain how the use of traits with high reliability and heritability for quantitative trait locus (QTL) analysis can be valuable even when specific endophenotypes are

uncertain. While all the specific mechanisms of HPA dysregulation associated with the bovine temperament model have yet to be determined, QTL analysis of temperament traits in beef and dairy cattle continues to point to aspects of the HPA, including: dopamine metabolism (Hiendleder et al., 2003) and the dopamine receptor (Gutiérrez-Gil et al., 2008; Glenske et al., 2011).

CHAPTER III

THE EFFECTIVENESS OF VASOPRESSIN AS AN ACTH SECRETAGOGUE IN CATTLE DIFFERS WITH TEMPERAMENT*

Introduction

The term temperament has been used to characterize behavioral responses of cattle to human-animal interactions (Burrow, 1997). If we assume that a fear response underpins animal reactions toward man, then a case could be made for those animals of poor temperament (i.e., individuals with a greater adverse reaction to human-handling) exhibiting a greater fear response in general. In addition to interactions with humans, fear responses of domestic animals may also arise from all or one of the following: social interactions; encounters with novel species and situations; or sudden stimuli that can be visual, auditory, or tactile in nature. Thus, temperament assessments may not only characterize an animal's response to human handling, but also reflect the relative ease of evoking an individual's fear response.

Fear is a well known stimulus of the hypothalamic-pituitary-adrenal (HPA) axis and has been widely studied since the adrenal glands were associated with the fear response in Cannon's (1932) depiction of the "fight or flight" reaction to stressors.

---

*Reprinted with permission from The effectiveness of vasopressin as an ACTH secretagogue in cattle differs with temperament by K.O. Curley, Jr., D.A. Neuendorff, A.W. Lewis, F.M. Rouquette, Jr., R.D. Randel, and T.H. Welsh, Jr., 2010. Physiol. Behav. 101:699-704, Copyright 2010 by Elsevier Inc.

As glucocorticoids are the primary mediators of the HPA response, serum or plasma concentrations of these steroids can be utilized as physiologic indicators of an ongoing stress response or biological quantifications of an individual's stress responsiveness. In cattle, glucocorticoids have been associated with anxiety-related behaviors (Bristow and Holmes, 2007) and novel-object avoidance (Van Reenen et al., 2005). Concerning temperament in the bovine model, elevated cortisol concentrations have been observed in cattle that exhibit an excitable temperament when compared to calmer animals (Fell et al., 1999; Curley et al., 2006). Additionally, data generated from exogenous stimulation of the HPA axis in cattle suggest a possible disparity in pituitary-adrenal response dynamics that is linked to temperament (Curley et al., 2008).

Vasopressin's (VP) role as an ACTH secretagogue was suggested in the early 1950s (McCann and Brobeck, 1954). Since then VP has been demonstrated to be an important component of the endocrine stress response (for review, see Antoni, 1993). Synthetic VP has been shown to induce increased circulating cortisol concentrations in cattle (Wagner and Oxenrider, 1972) and has been demonstrated to do so via increasing ACTH release from the pituitary gland (Senn et al., 1995). The method of pituitary challenge with exogenous VP has been subsequently utilized for investigation into functions of the bovine stress axis (Veissier et al., 1999; Carroll et al., 2007; Knights and Smith, 2007) and is well accepted. To date exogenous VP has not been incorporated into investigations of HPA function in the bovine temperament model. However, there is evidence to suggest that endogenous VP may play a role in the HPA dysfunction associated with fearfulness and anxiety as Aubry et al. (1995) observed increased basal

VP mRNA expression in the paraventricular nuclei (PVN) of RLA compared to RHA rats. Additionally, increased VP mRNA expression has been demonstrated in the PVN of both stressed and unstressed HAB rats compared to those of the less anxious line (Wigger et al., 2004).

The objective of the following experiment was to compare the pituitary and adrenal activity following pituitary stimulation with exogenous VP, in cattle exhibiting an excitable temperament and those of a calm temperament.

Materials and Methods

Experimental Design

All experimental procedures were in compliance with the Guide for the Care and Use of Agriculture Animals in Research and Teaching and approved by the Institutional Animal Care and Use Committee of Texas A&M University. A total of 9 crossbreed steers, approximately 1 year of age, were utilized to compare the pituitary and adrenal response to pharmacological stimulus of the pituitary gland, in calm and excitable cattle. Steer temperament was determined via exit velocity measures obtained at weaning, 4 months prior to the vasopressin challenge. Exit velocity (Burrow et al., 1988) is the rate at which the animals exited a squeeze chute and traversed a fixed distance (1.83 m). This measure specifically quantifies the relative degree of fear response generated by human handling of cattle (Burrow, 1997; Kilgour et al., 2006) and provides individual temperament appraisals that are consistent over time (Curley et al., 2006; King et al., 2006; Müller and von Keyserlingk, 2006). The calm (C) and excitable (E) groups consist

of the 5 slowest (EV= 1.69 ± 0.19 m/s) and 4 fastest (EV= 4.01 ± 0.2 m/s) individuals from a pool of 37 steers. Plasma cortisol concentrations collected at the time of EV assessment confirmed that adrenal activity of these individuals segregated with the temperament groups (C= 9 ± 1.6; E= 14 ± 1.4 ng/mL).

Throughout the duration of the experiment, except for the time periods of blood sampling, the steers were group-housed in a single pen (30 m x 10 m) and given free choice access to water and Coastal Bermuda grass hay. Each animal was fitted with a temporary indwelling jugular cannula, as described by Curley et al. (2008), 2 h prior to the initiation of the serial blood sampling that lasted for 12 h. Six and one half hours prior to VP treatment the steers were removed from the pen, haltered, and tied within segments of a chute system. Blood sampling at 0.25-h intervals, via the jugular catheters, initiated 6 h prior to vasopressin treatment and continued until 3 hours before vasopressin treatment when the blood sampling interval increased to 0.5 h. Midway through the blood sampling period a bolus dose consisting of 1.0 μg arginine^{8}-VP (Calbiochem, La Jolla, CA, Cat. #676435) per kg BW was administered via jugular catheter using physiological saline (0.9%) as the vehicle. Blood collections resumed at 5-min intervals until 1 h post vasopressin when collection intervals increased to 15 min and continued as such for 2 h. During the final 3 h of sampling, collection intervals increased to 30 min and remained constant until the final samples were collected 6 h post vasopressin treatment. Following collection of each sample, physiological saline solution followed by heparinized saline was infused to replace fluid volume and prevent blood clotting in the cannulas, respectively. Sample coagulation was prevented by use

of vacutainer tubes containing EDTA solution (BD, Franklin Lakes, NJ, Cat. #366457). Samples were immediately placed in an ice bath and centrifuged at 4 °C within 3 h of collection. Plasma samples were frozen and stored at −20 °C until ACTH and cortisol concentrations were analyzed via specific radioimmunoassays.

Radioimmunoassays

ACTH concentrations were determined in duplicate samples using a commercially available, double antibody RIA kit (MP Biomedicals, Orangeburg, NY, Cat. #07-106102). This RIA kit utilizes rabbit anti-porcine ACTH-conjugate as primary antiserum, standards made from human synthetic $ACTH^{1-39}$, $hACTH\text{-}125^{I}$ as the radio-labeled tracer and a secondary antiserum of goat anti-rabbit gamma-globulin. Counts per minute were obtained by using an automatic gamma counter (Micomedics Systems Inc., Horsham, PA) and unknown ACTH concentrations determined using Assay Zap software (Biosoft, Cambridge, UK). ACTH antiserum cross-reactivity was: $ACTH^{1-39}$, 100%; $ACTH^{1-24}$, 100%; hβ Lipotropin, 0.8%; and hα Lipotropin, 0.1%, (determined by MP Biomedicals). Interassay and intraassay CV were 7.8% and 9.0%, respectively.

Cortisol concentrations were determined from duplicate samples using a single antibody RIA procedure (Carroll et al., 2007) and utilized: rabbit anti-cortisol antiserum (Pantex, Div. of Bio-Analysis Inc., Santa Monica, CA, Cat. #P44) diluted 1:2500; standards made by serial dilution (8000 pg/100 μL to 3.9 pg/100 μL) of 4-pregnen-11β,17,21-triol-3,20-dione (Steraloids Inc., Newport, RI, Cat. #Q3880-000); and radio-labeled cortisol: 3H-Hydrocortisone (1,2-3H, NEN, Boston, MA, Cat. #NET-185).

Counts per minute (cpm) were obtained from a liquid scintillation spectrophotometric beta-counter (Beckman Coulter LS 6500) and unknown cortisol concentrations were calculated using Assay Zap software (Biosoft, Cambridge, UK). Cortisol antiserum cross-reactivity was: corticosterone, 60%; deoxycorticosterone, 48%; progesterone, 0.01%; and estradiol, 0.01%, (determined by Pantex). Interassay and intraassay CV were 8.3% and 12.9%, respectively.

Statistical Analysis

Repeated measures analysis, specific for mixed models, was conducted to determine the effects of time, temperament, and time x temperament on hormone concentrations throughout the duration of blood sampling. Within the statistical model a random effect from individual animals is accounted for. Additionally, the correlation between repeated samples, within each animal, is modeled using a heterogeneous autoregressive covariance structure. The GLM procedure of SAS (SAS Inst., Inc., Cary, NC) was utilized for ANOVA of temperament effects on pituitary and adrenal function parameters where repeated measures analysis was not appropriate. Such parameters included: baseline ACTH and cortisol concentrations, peak hormone concentrations, amplitude (calculated as the difference between peak and baseline concentrations) of the ACTH and cortisol response to challenge, time to return to baseline concentrations and areas under the hormone response curves. The areas under the response curves were determined utilizing the following equation:

AUC PRE = Σ ({(H_n + H_n + 1) / 2} • h); where H is the hormone concentration at a given time point and h is the time in hours between the two hormone concentrations.

Results

Cortisol concentrations as well as the dynamics of the circulating cortisol profiles over the 6 h prior to VP administration were markedly different between the two cattle temperament groups (Fig. 1). At the initiation of blood sampling plasma concentrations of cortisol in the excitable cattle (17 ± 3.9 ng/mL) were only numerically greater than that of the calmer animals (11 ± 0.9 ng/mL). Over the subsequent 3 h, the effects of time ($P < 0.01$), temperament ($P = 0.02$) and a time by temperament interaction ($P = 0.02$) affecting circulating cortisol concentrations were observed. In the calm cattle cortisol concentrations were on the decline 15 min following the onset of sample collection and continued to steadily decrease over the 2 h that followed. Conversely, elevated cortisol concentrations persisted in the excitable animals for one hour before beginning to decline. After about 2.5 h cortisol concentrations in both the calm and excitable groups stabilized and remained relatively unchanged for the duration of the pre-challenge sampling period. However, throughout the remaining 3 hours prior to VP administration, animals in the excitable group sustained circulating cortisol concentrations about two-fold higher than the calmer cattle.

Plasma ACTH concentrations increased following challenge with vasopressin and subsequently declined to concentrations equal to those observed in each of the temperament groups during the pre-challenge state (Fig. 2). For the assessment of either

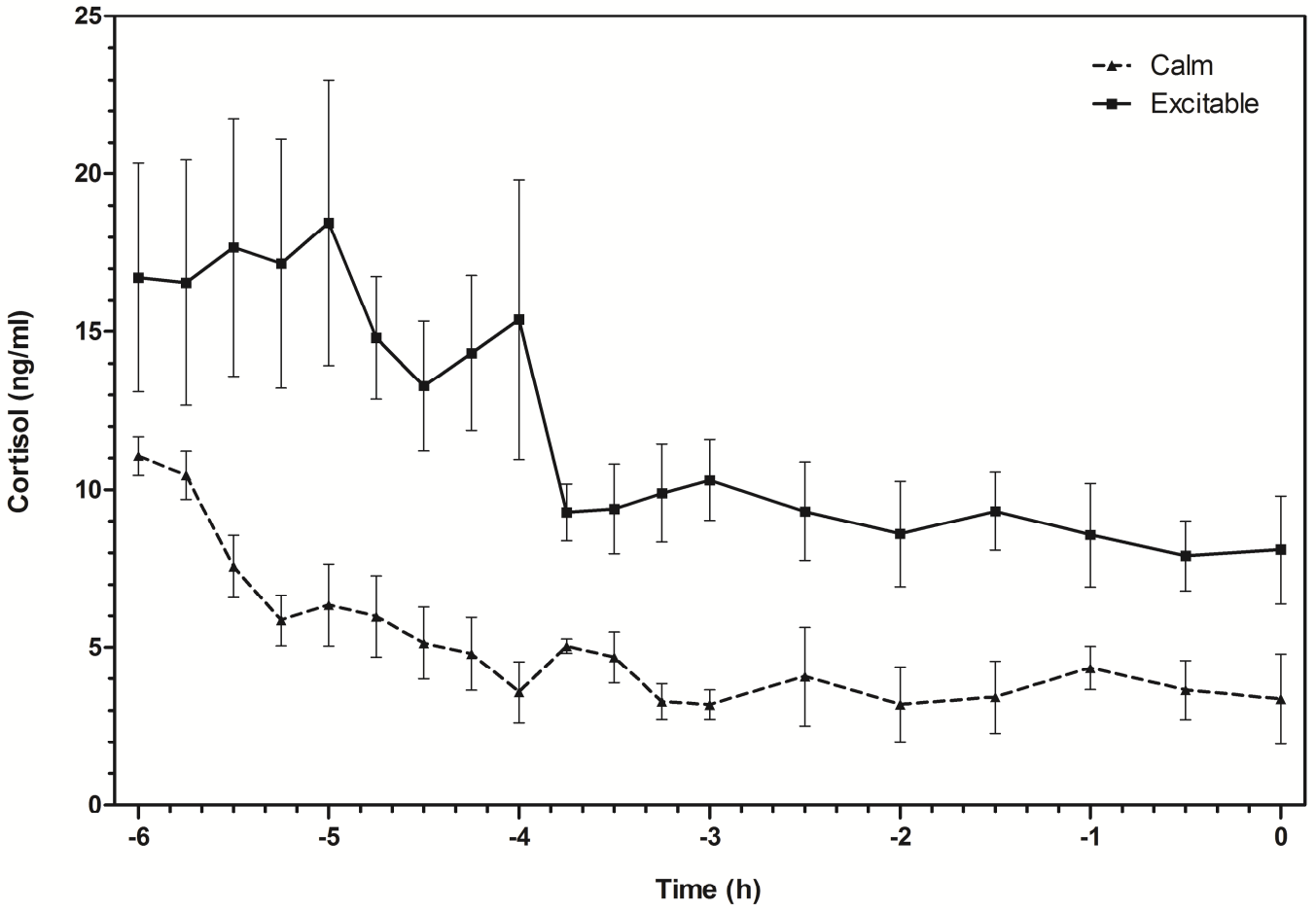

Figure 1. Mean plasma cortisol concentrations during the 6-h sampling period prior to stimulation with exogenous vasopressin for both calm (triangles) and excitable (squares) steers.

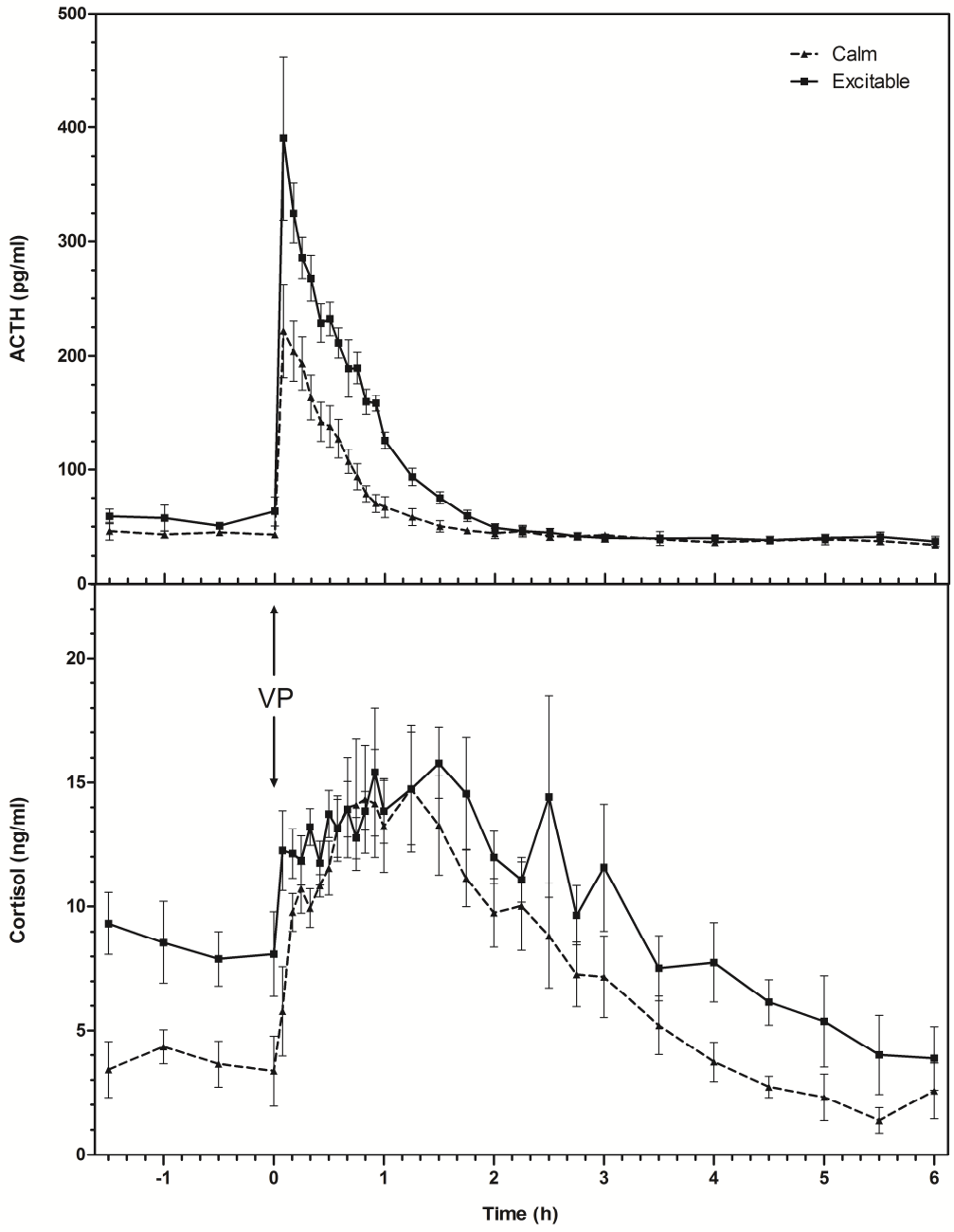

Figure 2. *Above:* Mean plasma ACTH concentrations following administration of exogenous vasopressin in calm (triangles) and excitable (squares) steers. *Below:* Mean plasma cortisol concentrations following administration of exogenous vasopressin in calm (triangles) and excitable (squares) steers. Arginine vasopressin administered at time 0.

pituitary or adrenal response to VP stimulus baseline hormone concentrations were determined as the pooled mean of hormone concentrations during the 1.5 h leading up to VP administration (a total of 4 plasma samples per animal). Baseline ACTH concentrations tended ($P \leq 0.05$) to differ between temperament groups with the excitable animals exhibiting numerically higher concentrations than the calmer steers (Table 1). During the initial one h post VP administration mean ACTH concentrations were influenced by time ($P < 0.0001$), temperament ($P = 0.001$), and exhibited a time by temperament interaction ($P < 0.001$). The dose of vasopressin induced a rapid increase in ACTH concentrations in all animals within the first 5 min post-challenge. This pituitary stimulation resulted in a greater ($P < 0.05$) peak ACTH output from the excitable when compared to the calm animals. In addition, the amplitude of the ACTH response was also larger ($P < 0.05$) in the excitable group. Within the first 2 h post-challenge, ACTH concentrations returned to baseline in both the calm and excitable cattle. In animals of both temperament groups the ACTH concentrations had already begun to decline from their peak at 10 min post-challenge. The time required to return to baseline concentrations was numerically greater in the excitable steers; however, the two groups did not statistically differ ($P = 0.3$) in this response parameter. The area under the ACTH curve during the first 2 h demonstrates a pituitary response to VP challenge of substantially greater ($P \leq 0.001$) magnitude in the excitable animals. These AUC data coupled with the amplitude of response and the peak ACTH concentrations may highlight a greater effectiveness of exogenous VP on the induction of pituitary function in the more excitable cattle compared to the calmer animals.

Table 1. Parameters of pituitary function following stimulation with exogenous arginine vasopressin measured in cattle of differing temperaments.

Response Parameter	**Calm (n =5)**	**Temperamental (n = 4)**
Baseline ACTH concentrations (pg/mL)	44 ± 1.7	57 ± 6.0†
Peak ACTH concentrations (pg/mL)	241 ± 33.2	414 ± 50.2*
Amplitude of ACTH response (pg/mL)	197 ± 33.2	357 ± 45.0*
Fold Increase	4.5 ± 0.8	6.3 ± 0.4
Return to Baseline (min)	108 ± 15	131 ± 17
AUC 2h Post Challenge (pg h/mL)	180 ± 14.4	297 ± 14.0***

†$P < 0.10$ * $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$

Cortisol concentrations (Fig. 2) over the first 3 h following VP challenge were not influenced by temperament ($P > 0.2$). However, a time by temperament interaction ($P < 0.01$) was evident during the first h of the post-challenge period. Prior to challenge, baseline cortisol concentrations (Table 2) were considerably lower ($P < 0.01$) in the calm cattle compared to the excitable animals. As a result of the exogenous VP both the peak cortisol concentrations reached and the amplitude of adrenal response did not differ ($P > 0.3$) with temperament. Although, there was a greater ($P < 0.02$) stimulation of adrenal activity in the calm cattle if we consider the fold-increase from their much lower baseline cortisol concentrations. Analysis of area under the cortisol curve illustrated no difference in total adrenal output ($P = 0.3$) between calm (32 ± 4.4 ng h/mL) and excitable (38± 3.6 ng h/mL) steers during the first 3 h post-challenge. Over the entirety of the 6-h post-challenge sampling period the AUC of mean cortisol concentrations was numerically greater ($P = 0.17$) in the excitable cattle, which is most likely evidence of a return to divergent baseline concentrations rather than evidence of VP stimulation of the pituitary. The time to return to baseline cortisol concentrations was significantly ($P < 0.01$) shorter in the excitable cattle compared to the calm animals, but this is likely an artifact of the baseline values being substantially higher in the excitable animals and may not be physiologically relevant. Repeated measures analysis of variance revealed a tendency for temperament ($P < 0.08$) to influence cortisol concentrations during the final 3 h post-challenge. It is during this time that cortisol concentrations in the calm and excitable steers diverged as they returned to profiles similar to those observed during the 3 h prior to VP administration.

Table 2. Parameters of adrenal function following stimulation with exogenous arginine vasopressin measured in cattle of differing temperaments.

Response Parameter	**Calm (n =5)**	**Temperamental (n = 4)**
Baseline Cortisol concentrations (ng/mL)	4 ± 0.5	8 ± 1.2**
Peak Cortisol concentrations (ng/mL)	15 ± 2.3	17 ± 2.1
Amplitude of Cortisol response (ng/mL)	12 ± 2.1	8 ± 2.6
Fold Increase	3.3 ± 0.5	1.1 ± 0.4*
Return to Baseline (min)	228 ± 15	165 ± 6*
AUC 6h Post Challenge (ng h/mL)	43 ± 6.7	58 ± 6.0

$^{\dagger}P < 0.10$ * $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$

Discussion

With all animal models utilized for investigation of HPA function, researchers must always take into account the effects of the experimental procedure's augmentation of the endocrine stress axis. Employment of the bovine temperament model requires additional consideration of this fact as a subset of the research animals have been specifically selected for their increased responsiveness to handling by humans. The cortisol profiles during the initial hours of blood sampling demonstrate that the HPA axis of the calm as well as the excitable animals responds to the human-animal interactions required for catheterization and situation of the cattle within the chute system. Additionally, as is to be expected, both the magnitude and duration of this initial adrenal response were significantly greater in the more excitable animals. Fear is a stressor of great significance and can be manifested by a strong cortisol response to handling in cattle with excitable dispositions (Grandin, 1997). While the placement of the catheters undoubtedly caused a physiologic stress response in these cattle, the bulk of the observed elevation in initial cortisol concentrations is probably due to the handling of the animals. The use of similar sampling procedures (Curley et al., 2008), but incorporating catheterization 18 h prior to the initiation of blood sampling, also induced an initial stress response similar in pattern to that which was observed in this study. Within about 2 h from the onset of blood sampling the initial adrenal response seemed to have subsided as the animals became acclimated to the experimental surroundings as well as to the persistent presence of human handlers. Over the remaining 4 h of the pre-challenge period the mean cortisol concentrations remained unchanged relative to

temperament group; however, the baseline adrenal output of the excitable cattle remained almost double that of their calmer counterparts. At this juncture it remains unclear if the higher baseline cortisol concentrations are resultant from continued stress placed on the excitable animals by the experimental environment or to elevated basal adrenal function inherently associated with temperament. As one considers the post challenge data generated from this experiment, the differing HPA activity that occurred 6 h prior to VP administration as well as the dissimilar baseline cortisol concentrations in the calm and excitable groups must not be dismissed.

As evidenced by multiple ACTH response parameters presented herein, stimulation with exogenous VP resulted in greater pituitary activation in cattle with an excitable temperament. Interestingly, we observed just the opposite in a previous study (Curley et al., 2008) where cattle of calm temperament exhibited a greater pituitary response to exogenous bCRH. When comparing the effectiveness of equimolar dosages of CRH and VP, CRH stimulated bovine corticotrophs in culture to release greater amounts of ACTH (Carroll et al., 2007). The caveat that must be acknowledged before further discussion of the data from this experiment in conjunction with the previous CRH challenge data (Curley et al., 2008), is that the CRH dosage used was comparable to the physiologic range while the VP dose in this study likely resulted in a stimulus approaching pharmacological proportions. While this fact may provide explanation for the greater overall magnitude of ACTH response observed in all animals stimulated with VP, it does not seem to provide clarification for the opposite effects of temperament on pituitary response elicited by each trophic agent.

Negative feedback mechanisms of glucocorticoids on corticotroph function may underpin our observed discrepancies in pituitary response induction between CRH and VP, as circulating corticosteroids are paramount in HPA regulation (Keller-Wood and Dallman, 1984). Pre-challenge adrenal activities were of similar patterns in both this experiment and the previous CRH challenge (Curley et al., 2008). Additionally, in both studies cattle of an excitable temperament exhibited an increased initial adrenal responsiveness to handling followed by an acclimation that yielded baseline cortisol concentrations greater than those in cattle of calm temperament. This hypercoritsolemic condition of the excitable cattle may have altered pituitary responsiveness of those animals. Cortisol perfusion has been reported to have a greater inhibition of CRH-induced rather than VP-induced ACTH release from equine pituitary cells (Evans et al., 1993). Chronic stress in pigs does not seem to alter the sensitivity of the pituitary to VP stimulus (Janssens et al., 1995). Additionally, the general consensus from work in the rat is that combined CRH/VP stimulation of corticotrophs is less susceptible to inhibition by corticosterone than stimulation with CRH alone (Dallman, 1993). Finally, chronic restraint stress has been shown to increase VP receptor mRNA expression (Rabadan-Diehl et al., 1995) yet reduce CRH receptor number (Hauger et al., 1988) in the adenohypophysis. It is most likely through these mechanisms that the responsiveness of the HPA axis is preserved even during periods of chronic stress (Aguilera and Rabadan-Diehl, 2000). While it remains unclear if cattle of an excitable temperament are in a state of chronic stress, the data generated using differing ACTH secretagogues does adhere to the expected pituitary responses during a state of hypercortisolemia. Although, an

alternative argument that attributes the greater pituitary response to VP as a byproduct of elevated adrenal activity associated with handling stress at the onset of blood sampling could also be made. However, Knights and Smith (2007) demonstrated that transportation stress prior to a VP challenge *in vivo* resulted in a blunted ACTH response even in the presence of up-regulated VP receptor mRNA expression in the bovine pituitary.

There is a growing body of evidence that indicates a potentially significant role for vasopressin in the physiology associated with psychological disorders such as depression (for review, see Dinan and Scott, 2005). While the data generated from this and previous endocrine challenges (Curley et al., 2008) within the bovine temperament model fail to parallel models of depression in terms of adrenal response to VP (Dinan and Scott, 2005), our findings of altered pituitary function associated with excitable temperament do agree with the enhanced ACTH response to VP (Dinan et al., 2004) and blunted response to pituitary stimulation with CRH (Gold and Chrousos, 1985) observed in depressed human patients. By using a temperament selection criterion, cattle with a hyper-responsive HPA axis can be identified via their individual behavioral responses to handling. This bovine temperament model may be of use to both decipher mechanisms associated with HPA dysfunction and to elucidate physiological phenotypes or pathologies that have parallels in other species.

CHAPTER IV

THE INTERPLAY BETWEEN TEMPERAMENT AND BREEDTYPE ON THE BOVINE HYPOTHALAMIC-PITUITARY-ADRENAL (HPA) AXIS

Introduction

Dissimilarity in the relative ease of handling across many breeds of cattle is widely acknowledged by producers and agricultural scientists alike. As early as cattle temperament has been scientifically characterized, differences in temperament of various breedtypes have been observed (Tulloh, 1961). Of particular significance are the multitude of breed comparisons that document the great distinction between temperament of *Bos taurus* and *Bos indicus* breeds. In general, Brahman cattle are more excitable and become more stressed when compared to English breeds such as Angus (Grandin, 1980). Fordyce et al. (1982) reported that Brahman cattle had much poorer temperament measures than the English breeds. Previously, Hearnshaw et al. (1979) demonstrated that as little as ¼ influence of the Brahman genotype resulted in offspring that had substantially poorer temperament scores and thus were associated with a higher degree of handling difficulty. Although there has been much description of the behavioral differences across cattle breeds, there has been little investigation into the biological ramifications of said differences. Whereas differences in physiological dynamics of the hypothalamic-pituitary-adrenal (HPA) axis attributed to cattle temperament have been confirmed (Curley et al., 2008), there has yet to be an effort to discern the impact of breed on this component of the endocrine system. However, there

is evidence that genetic strains of rats differ with regard to behavior, stress responsiveness, and HPA function (Dhabhar et al., 1997; Windle et al., 1998; Moncek et al., 2001).

The objective of the following experiment was to compare the pituitary and adrenal activity following pituitary stimulation with exogenous corticotrophin-releasing hormone (CRH), in steers of differing temperament, and of breedtypes that have been historically recognized as either highly excitable or generally calm by comparison.

Materials and Methods

Animals

The steers in this study represented the temperament extremes from two separate herds. The Brahman (B) cattle were part of a single calf crop from the Texas AgriLife Research Center in Overton, TX; whereas, the Angus (A) cattle were part of a single calf crop from the Mississippi Agricultural and Forestry Experiment Station in Raymond, MS. All animals were spring-born and weaned approximately 6 mo later. Temperament was determined via exit velocity (EV) which is the rate that the animals exit a squeeze chute and traverse a fixed distance of 1.83 m (Burrow et al., 1988). This measure specifically quantifies the relative degree of fear response generated by human handling of cattle (Burrow, 1997; Kilgour et al., 2006) and provides individual temperament appraisals that are consistent over time in beef (Curley et al., 2006; King et al., 2006; Müller and von Keyserlingk, 2006) and dairy (Gibbons et al., 2011) cattle.

For this study, EV was measured at weaning and the calm and excitable groups consisted of the 8 slowest (B = 1.25 ± 0.25; A = 2.43 ± 0.23 m/s) and 8 fastest (B = 2.28 ± 0.46; A = 3.25 ± 0.16 m/s) steers respectively. Then about 2 mo after weaning, the Angus steers were shipped to Overton and both Brahman and Angus steers were maintained as one group on winter pasture. Over the subsequent 3 mo, all animals were handled at 28-d intervals in order to obtain BW measurements.

Experimental Design

All of the following procedures were reviewed and approved by the Texas A&M University Institutional Animal Care and Use Committee (AUP 2003-63). In order to compare pituitary–adrenal activity following pituitary stimulation, all steers were utilized for a CRH challenge experiment at approximately 1 year of age (BW = 288 ± 17.4 kg). Throughout the duration of the experiment, except for the time periods of blood sampling, the steers were penned together and given free choice access to water, rye-grass pasture, and Coastal bermudagrass hay. On challenge days, 4 animals (2 B and 2 A) were confined, but not restrained within segments of a working chute for a period of 12 h. Midway through this period a bolus of 0.1 μg bCRH (Peninsula Laboratories, San Carlos, CA, Cat. #8568) per kg BW was administered via jugular cannula using physiological saline (0.9%) as the vehicle, with BW determined at the time of cannulation. Each animal was fitted with an indwelling jugular catheter, as described by Curley et al. (2008), 18 h prior to the initiation of serial blood sampling.

Six-and-one-half h prior to corticotrophin-releasing hormone treatment the steers were removed from the pen, haltered, and tied within segments of a chute system. Blood sampling at 0.25-h intervals, via the jugular catheters, commenced 6 h prior to CRH treatment and continued until 3 h pre-CRH where the blood sampling interval increased to 0.5 h. Following the administration of CRH, blood collections resumed at 5-min intervals until 1 h post CRH; then collection intervals increased to 15 min and continued as such for 2 h when the intervals increased to 30 min and remained constant until the final samples were collected 6 h post CRH treatment. At this point the catheters were removed and the animals returned to the pasture.

After collecting each sample, physiological saline solution followed by heparinized saline was infused to replace fluid volume and prevent blood clotting in the cannulas, respectively. Sample coagulation was prevented by use of vacutainer tubes containing EDTA solution (BD, Franklin Lakes, NJ, Cat. #366457). Samples were immediately placed in an ice bath and centrifuged at 4 °C within 3 h of collection. Plasma samples were frozen and stored at −20 °C until ACTH and cortisol concentrations were analyzed via specific radioimmunoassay. Additionally, separate aliquots were snap-frozen in liquid nitrogen and stored at -80 °C until catecholamine concentrations were measured via enzyme immunoassay.

Immunoassays

ACTH concentrations were determined in duplicate samples using a commercially available, double antibody RIA kit (MP Biomedicals, Orangeburg, NY,

Cat. #07-106102). This RIA kit utilizes rabbit anti-porcine ACTH-conjugate as primary antiserum, standards made from human synthetic $ACTH^{1-39}$, $hACTH-^{125}I$ as the radio-labeled tracer and a secondary antiserum of goat anti-rabbit gamma-globulin. Counts per minute were obtained by using an automatic gamma counter (Micomedics Systems Inc., Horsham, PA) and unknown ACTH concentrations determined using Assay Zap software (Biosoft, Cambridge, UK). ACTH antiserum cross-reactivity was: $ACTH^{1-39}$, 100%; $ACTH^{1-24}$, 100%; hβ Lipotropin, 0.8%; and hα Lipotropin, 0.1%, (determined by MP Biomedicals). Interassay and intraassay CV were 8.5% and 10.2%, respectively.

Cortisol concentrations were determined from duplicate samples using a single antibody RIA procedure (Carroll et al., 2007) and utilized: rabbit anti-cortisol antiserum (Pantex, Div. of Bio-Analysis Inc., Santa Monica, CA, Cat. #P44) diluted 1:2500; standards made by serial dilution (8000 pg/100 μL to 3.9 pg/100 μL) of 4-pregnen-11β,17,21-triol-3,20-dione (Steraloids Inc., Newport, RI, Cat. #Q3880-000); and radio-labeled cortisol: ^{3}H-Hydrocortisone (1,2-3H, NEN, Boston, MA, Cat. #NET-185). Counts per minute (cpm) were obtained from a liquid scintillation spectrophotometric beta-counter (Beckman Coulter LS 6500) and unknown cortisol concentrations were calculated using Assay Zap software (Biosoft, Cambridge, UK). Cortisol antiserum cross-reactivity was: corticosterone, 60%; deoxycorticosterone, 48%; progesterone, 0.01%; and estradiol, 0.01%, (determined by Pantex). Interassay and intraassay CV were 5.1% and 7.9%, respectively.

Catecholamine concentrations were determined from duplicate samples using a commercially available ELISA kit (Alpco Diagnostics, Boston, MA, Cat. # 17-BCTHU-

E02) previously used in our laboratory (Burdick et al., 2010; Burdick et al., 2011b). The protocol was carried out as per manufacturer's direction. Assay Zap software (Biosoft, Cambridge, UK) was used to calculate unknown catecholamine concentrations from absorbance data. The lower limit of assay detection sensitivity was 10 pg/mL for epinephrine and 50 pg/mL for norepinephrine. Interassay and intraassay CV were 8.3% and 8.9%, respectively.

Statistical Analysis

Repeated measures analysis, specific for mixed models, was conducted to determine the effects of time, temperament, and time x temperament on hormone concentrations throughout the duration of blood sampling. Within the statistical model a random effect from individual animals is accounted for. Additionally, the correlation between repeated samples, within each animal, was modeled using a heterogeneous autoregressive covariance structure. The GLM procedure of SAS (SAS Inst., Inc., Cary, NC) was utilized for ANOVA of temperament, breedtype, and temperament x breedtype effects on pituitary and adrenal function parameters where repeated measures analysis was not appropriate. Such parameters included: baseline ACTH and cortisol concentrations, peak hormone concentrations, amplitude (calculated as the difference between peak and baseline concentrations) of the ACTH and cortisol response to challenge, areas under the hormone response curves. The areas under the response curves were determined utilizing the following equation:

AUC RESP = Σ [({(Hn + Hn + 1) / 2} • h) – BASLINE • h]; where H is the hormone concentration at a given time point, h is the time in hours between the two hormone concentrations, and BASELINE is the mean concentration over the final hour of sampling prior to challenge.

Results and Discussion

At the onset of experimental procedures, the initial handling of the animals resulted in adrenal activity that seems to have been influenced by both temperament and breedtype (see Table 3). Catecholamine concentrations were markedly higher ($P < 0.05$) in the excitable Angus steers when compared to the calm steers of either breed. Plasma concentrations of epinephrine and norepinephrine in the excitable Brahman steers were only higher ($P < 0.02$) than those measured in the calm Brahman steers when analyzed independent of the Angus steers. Elevated sympathoadrenal medullary function in the more excitable animals conforms with the overall paradigm of cattle temperament and stress responses to handling. Similarly, Burdick and colleagues recorded a temperament-related effect on adrenal-medullary secretion associated with transportation stress (2010) and the handling linked to experimental procedures of an endotoxin challenge (2011b).

Table 3. Adrenal function at the initiation of challenge procedures (6 h prior to CRH challenge) in both Angus and Brahman steers of differing temperament.

	Angus		Brahman	
Parameters of Adrenal Hormones	**Calm (n =8)**	**Excitable (n = 8)**	**Calm (n =8)**	**Excitable (n = 8)**
Initial **Cortisol** concentrations (ng/mL)	31 ± 3.0[a]	44 ± 5.7[b]	26 ± 3.2[a]	28 ± 4.2[a]
Initial **Epinephrine** concentrations (pg/mL)	299 ± 96.6[a]	713 ± 178.3[b]	168 ± 24.0[a]	449 ± 104.3[a,b]
Initial **Norepinephrine** concentrations (pg/mL)	333 ± 53.7[a]	715 ± 200.5[b]	327 ± 34.0[a]	547 ± 67.2[a,b]

[a-b] Within a row, LS means without a common superscript differ ($P < 0.05$)

This pattern of higher adrenal output in the excitable animals wasn't as straightforward when glucocorticoids were measured. While the mean cortisol concentrations sampled at the onset of experimental procedures were considerably greater ($P < 0.04$) when comparing the excitable Angus steers to any of the three other animal groups, there was not; however, a temperament-related difference ($P = 0.6$) in cortisol concentrations amongst the Brahman animals during this time.

Six h after the initiation of blood sampling, all animals received a bolus of CRH; the pituitary response from which is depicted (Fig. 3) and detailed (Table 4) below. While these data portray a similar pituitary ACTH response across breed and temperament group, there are a few noteworthy details within them. Baseline concentrations (i.e. average during the hour prior to administering CRH) of ACTH were similar across all groups, with the exception of the excitable Angus steers exhibiting higher ($P < 0.05$) concentrations than both the Angus and Brahman groups of calm steers. As was the case six h prior, at the initiation of experimental handling, the HPA axis was still being stimulated in the Angus steers either from the experimental surroundings or something inherently associated with their temperament.

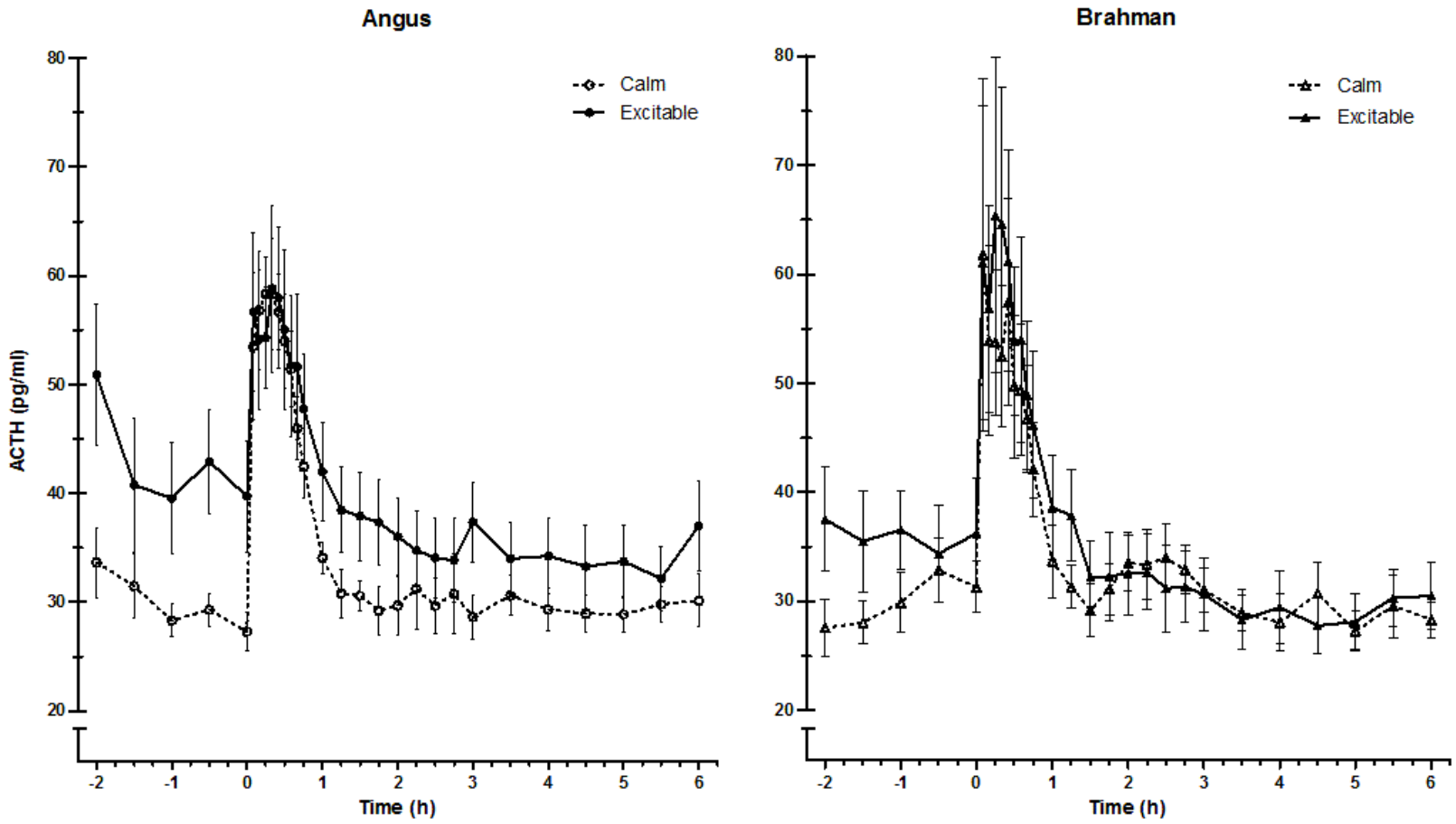

Figure 3. Mean plasma concentrations of ACTH following administration of exogenous corticotrophin-releasing hormone (CRH) in calm (open) and excitable (closed) Angus (circles) and Brahman (triangles) steers. CRH administered at time 0.

Table 4. Parameters of pituitary function prior to, and following stimulation with exogenous CRH in both Angus and Brahman steers of differing temperament.

	Angus		Brahman	
Response Parameter	**Calm (n =8)**	**Excitable (n = 8)**	**Calm (n =8)**	**Excitable (n = 8)**
Baseline ACTH concentrations (pg/mL)	28 ± 1.5[a]	41 ± 4.8[b]	31 ± 2.5[a]	36 ± 4.2[a,b]
Peak ACTH concentrations (pg/mL)	66 ± 4.9[a]	65 ± 7.5[a]	69 ± 15.1[a]	72 ± 14.7[a]
Amplitude of ACTH response (pg/mL)	37 ± 5.0[a]	24 ± 5.1[a]	38 ± 17.2[a]	36 ± 11.5[a]
AUC RESP 2 h Post Challenge (pg h/mL)	31 ± 3.9[a]	20 ± 4.2[a]	26 ± 9.0[a]	27 ± 6.0[a]

[a-b] Within a row, LS means without a common superscript differ ($P < 0.05$)

The elevated pituitary output of the excitable Angus steers translated to increased baseline adrenal function as well (see Table 5 and Figure 4). As was observed with baseline ACTH, epinephrine concentrations at the time of challenge were also greater ($P < 0.033$) in the excitable Angus groups when compared to calm animals of either breedtype. Moreover, baseline cortisol concentrations were markedly higher ($P < 0.004$) in the excitable Angus animals than in any of the other three experimental groups. Interestingly, there wasn't a difference linked to temperament in baseline pituitary function in the Brahman cattle at this time. An effect of temperament on baseline adrenal activity was evident ($P < 0.01$) in a separate analysis of only the Brahman groups.

In response to CRH, pituitary activity did not differ with regards to breedtype and temperament. Peak CRH-induced ACTH concentrations were comparable across all groups of steers. Similarly, the amplitude of the ACTH response didn't differ ($P > 0.4$) between any of the experimental groups. However, when comparing the Angus animals separately, the excitable steers demonstrated slightly smaller amplitudes of ACTH output in response to CRH stimulation. Additionally, while there was no statistical difference across all animal groups, in terms of pituitary activity measured by area under the curve, the excitable Angus steers also exhibited a blunted response compared to their calmer counterparts. These temperament–related nuances in amplitude and AUC of the pituitary response are similar to those observed in previous CRH challenges conducted on Brahman heifers (Curley et al., 2008). Yet, in this experiment, no such observations were evident in the Brahman steers but were found in the Angus steers.

Table 5. Parameters of adrenal function prior to, and following stimulation with exogenous CRH in in both Angus and Brahman steers of differing temperament.

	Angus		Brahman	
Response Parameter	**Calm (n =8)**	**Excitable (n = 8)**	**Calm (n =8)**	**Excitable (n = 8)**
Epinephrine at Challenge (pg/mL)	110 ± 21.8[a]	244 ± 62.9[b]	120 ± 10.4[a]	163 ± 38.9[a,b]
Norepinephrine at Challenge (pg/mL)	184 ± 37.5[a]	210 ± 21.6[a]	232 ± 48.1[a]	217 ± 25.9[a]
Baseline Cortisol concentrations (ng/mL)	10 ± 2.4[a]	24 ± 4.0[b]	7 ± 1.1[a]	13 ± 1.6[a]
Peak Cortisol concentrations (ng/mL)	35 ± 2.7[a]	37 ± 2.3[a]	28 ± 1.9[b]	28 ± 2.0[b]
Amplitude of cortisol response (ng/mL)	24 ± 3.4[a]	15 ± 3.4[b]	21 ± 2.5[a,b]	15 ± 2.3[b]
AUC RESP 2 h Post Challenge (ng h/mL)	26 ± 3.7[a]	15 ± 3.1[b]	23 ± 3.5[a,b]	15 ± 3.1[b]
Cortisol to ACTH AUC RESP Ratio	0.88 ± 0.12[a,b]	0.82 ± 0.2[a,b]	1.4 ± 0.36[b]	0.64 ± 0.08[a]

[a-b] Within a row, LS means without a common superscript differ ($P < 0.05$)

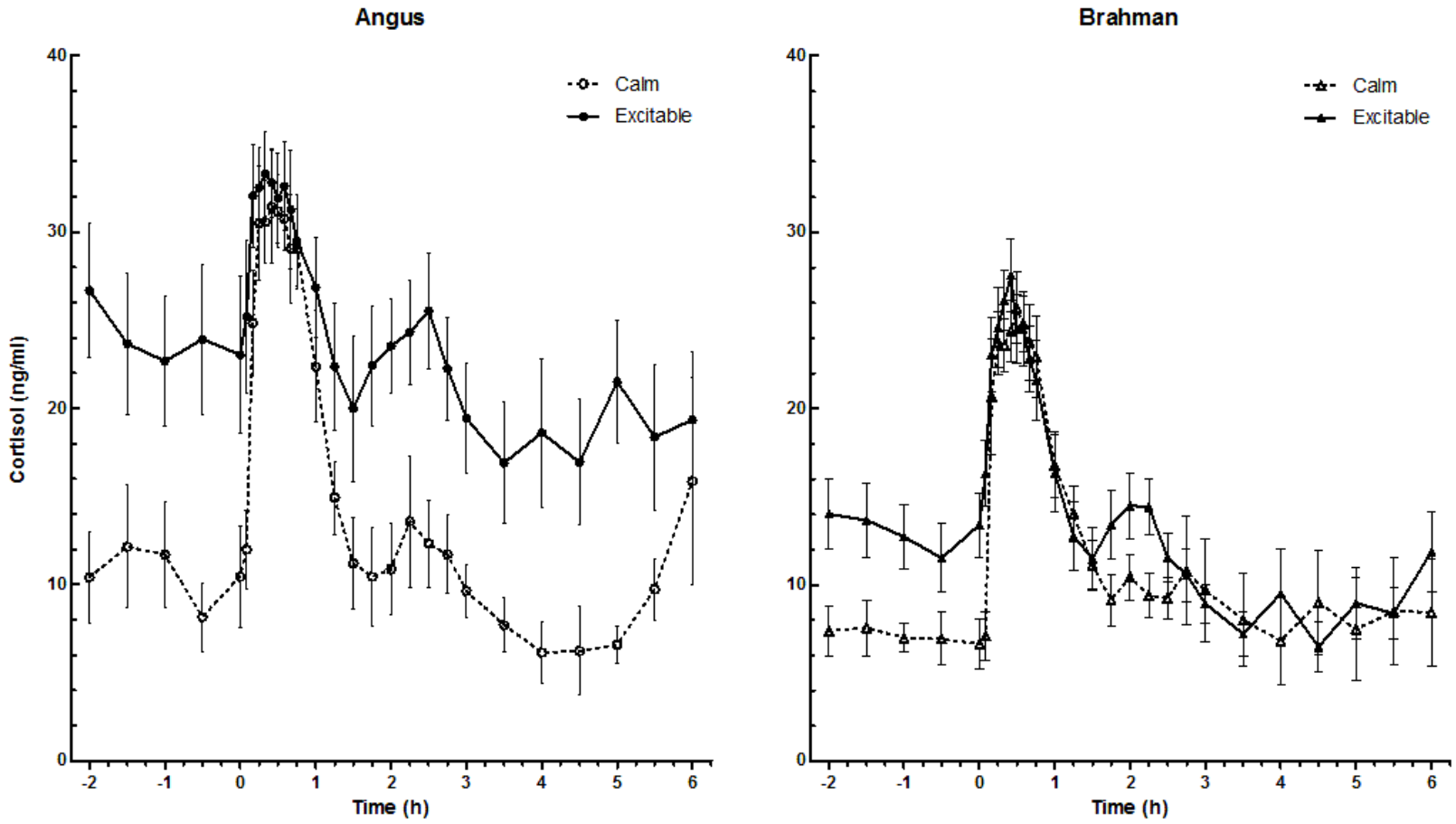

Figure 4. Mean plasma concentrations of cortisol following administration of exogenous corticotrophin-releasing hormone (CRH) in calm (open) and excitable (closed) Angus (circles) and Brahman (triangles) steers. CRH administered at time 0.

In terms of adrenal activity following exogenous CRH, an effect of temperament was apparent in only the Angus steers. While peak cortisol concentrations did not differ between calm and excitable cattle, the stimulated adrenal activity, measured as amplitude of cortisol response or area under the curve, was lessened ($P < 0.05$) in the more temperamental animals. Again, these observations agree with previous data from both CRH and ACTH challenges (Curley et al., 2008). This interplay of temperament and adrenal activity was not congruent with respect to the Brahman steers. Specifically, calm and excitable Brahman steers did not differ in peak cortisol concentrations ($P = 0.88$), amplitude of cortisol response ($P = 0.14$), or area under the post-challenge curve ($P = 0.10$). The only significant effect of temperament on response parameters, in the Brahman cattle, was represented by the ratio of cortisol and ACTH areas under the curve; which was markedly lower ($P < 0.02$) in the excitable animals. Incidentally, this difference wasn't observed when comparing the Angus steers of differing temperaments.

Habituation to handling and facilities may be one explanation why parallels to previous endocrine challenges that incorporated this temperament paradigm (Curley et al., 2008; Curley et al., 2010) were not observed in these particular Brahman cattle as they were with the Angus steers. These Brahman steers received more frequent human-animal interactions. During the 3 months after weaning, data collection for another study (not related to the experiment described herein) required frequent weighing of the Brahman herd. As a result, the Brahman steers utilized for our experiment, were handled 15 times over the 11 weeks between weaning and when the Angus steers arrived to the Overton ranch. The impact of rearing environment on animal behavior is evident

in findings of cattle that were more accustomed to handling, reacted less to humans (Boissy and Bouissou, 1988) and, in general, were easier to manage (Boivin et al., 1994). Also, habituation has been reported to reduce the cortisol response to handling in Brahman cattle (Andrade et al., 2001).

There have been conflicting reports on the specific impact of habituation to management facilities or acclimation to human handlers on exit velocity and adrenal function. A slight increase in exit velocity (i.e. an indication of worsened temperament) was observed over the course of 3 measurements, taken at 4-week intervals, in heifers that were group-housed in single pen (Müller and von Keyserlingk, 2006). Similarly, repeated measurements of 2-year-old steers have also indicated a rise in exit velocity during the first 80 days in the feedlot (Petherick et al., 2002). However, Cooke et al. (2009) didn't find an appreciable effect of acclimating cattle to humans, through bi-weekly sessions of hand-feeding while in a pasture, on measures of exit velocity or cortisol concentrations. Yet in an experiment specifically focused on the impact of either positive or negative handling experiences on cattle temperament, repeated handling resulted in slower exit velocity regardless of the quality of handling (Petherick et al., 2009a). Moreover, when the repeated handling wasn't poor (e.g. handlers making loud noise and slapping the animals) it resulted in lower cortisol concentrations than observed in the minimally handled steers (Petherick et al., 2009b).

While we do not have data to compare the HPA response to handling before and after the Brahman cattle received extensive interaction with people, the lack of distinction between these calm and excitable steers in regards to adrenal parameters at

the onset of serial blood sampling (Table 3) and baseline ACTH concentrations (Table 4) may be evidence of an acclimation to our working facilities and herdsmen. But even with this increased amount of human contact, exit velocity measured just days before the CRH challenge remained distinct ($P < 0.0001$) between the Brahman temperament groups (calm = 1.77 ± 0.24; excitable = 3.30 ± 0.23 m/s).

Sex-related differences in HPA function could be another possible explanation as to why the endocrine response to CRH challenge of the Brahman steers used herein didn't match previous observations from Brahman heifers (Curley et al., 2008). Differences HPA axis dynamics have been attributed to sex in a variety of species and in response to various stressors (for review, see Kudielka and Kirschbaum, 2005). Specifically, higher concentrations of glucocorticoids have been observed in female rats (Kitay, 1961), heifers (Henricks et al., 1984) and women (Kirschbaum et al., 1999). Agado and colleagues (2009) demonstrated higher basal concnetrations of cortisol and increased adrenal responsiveness to ACTH challenge in Brahman heifers compared to Brahman bulls. It is conceivable that the observations from the Brahman steers of this study reflect this sex-related paradigm.

Interestingly, the observations specific to the Brahman cattle may suggest a temperament-related endophenotype that was previously hidden. That is, the disparity in ratio of cortisol to ACTH areas under the curve could indicate inefficiency in cattle HPA function associated with an excitable temperament. To elaborate, it has been well-established that the bovine temperament model identifies cattle with an increased adrenal response to handling when compared to their calmer counterparts (Fordyce et al., 1988a;

Fell et al., 1999; Curley et al., 2006; Burdick et al., 2010; Cafe et al., 2011b). As glucocorticoids play an integral role in feedback regulation of the HPA axis (Dallman et al. 1992) and elevated cortisol concentrations have been shown to reduce pituitary response to stress stimuli (Dallman and Jones, 1973; Rivier and Vale, 1987), we have previously speculated that the observation of a blunted ACTH response to exogenous CRH challenge could be explained with this paradigm (Curley et al., 2008). An idea that the data from the Angus steers in this study would reinforce. However, either because of repeated handling or sex-linked mechanisms of HPA function, a temperament-based adrenal responsiveness was not present at the onset of the serial sampling of blood. As a result, in the excitable Brahman steers there was no blunting of pituitary responsiveness to our challenge with exogenous CRH used in this study. Although the stimulated concentrations of ACTH were similar between the calm and excitable Brahman steers, the ratio of cortisol to ACTH areas under the response curve was distinct and could be indicative of a novel type of HPA dysfunction linked to temperament. As such, specific focus on this aspect of temperament and pituitary-adrenal dynamic is warranted in further research.

A disparity in effects of temperament wasn't the only anomaly unveiled by comparing the Angus and Brahman cattle. Following the CRH challenge, peak concentrations of cortisol were notability higher ($P > 0.002$) in the Angus steers regardless of temperament. Although the literature is replete with indications of differences in temperament and HPA function between these two breedtypes, typically it is *Bos indicus*-crossed animals that exhibit elevated adrenal activity and poorer

temperament. Angus calves were shown to have lower concentrations of cortisol than Angus x Brahman calves in samplings before and after shipping (Blecha et al., 1984). Additionally, calves with a Brahman genetic component responded greater to stress associated with typical management practices (e.g. handling, weaning, social reorganization, and transport) than did Angus-crossed calves (Zavy et al. 1992). And although their study was primarily focused on comparing heat tolerance, Hammond and colleagues (1996) did observe lower concentrations of circulating cortisol in Angus heifers compared to the Brahman counterparts.

As for temperament, *Bos indicus*-influenced cattle have often been reported to be more easily excitable (Hearnshaw and Morris, 1984; Fordyce et al., 1988a; del Campo et al., 2010) and thus more difficult to work with, whereas *Bos taurus* cattle have been typically regarded with the reverse stereotype. Recently, findings from a multi-year study, containing over 3,000 head of cattle, indicated Angus-crossed calves as having a more favorable temperament when compared to a myriad of other European beef breedtypes (Hoppe et al., 2010). In a direct comparison, Cafe et al. (2011a) observed exit velocity measures and chute scores (a subjective assessment of cattle temperament) to be consistently lower in Angus than in Brahman cattle. However, in this study our data convey the opposite. Exit velocity measures at the time of weaning were faster ($P = 0.001$) in the Angus cattle (B= 1.76 ± 0.29; A= 2.84 ± 0.17 m/s). These breed differences in temperament manifested as differences in adrenal function 6 months later during our endocrine challenge. As evident by the Angus steers having higher ($P < 0.02$) concentrations of cortisol during our initial handling of the animals (B = 27 ± 2.6; A =

37 ± 3.5 ng/mL). And while these findings would seem to conflict with well-established breedtype norms, it would be incorrect to arrive at this conclusion. The Brahman and Angus steers used in this study represented the temperament extremes of two distinct calf crops originating from different ranches. Differences in EV measures and HPA responsiveness did not reflect inherent differences in temperament, but instead reaffirm the notion that temperament measures are relative to a specific population of cattle. Caution should be used when trying to compare measures of individual animal temperament from distinct ranches.

CHAPTER V

USING DEX/CRH CHALLENGE TO IDENTIFY HPA DYSREGULATION ASSOCIATED WITH CATTLE TEMPERAMENT

Introduction

The combination of a corticotrophin-releasing hormone (CRH) challenge following a dexamethasone suppression test (DST) is a common clinical tool used to diagnose hypothalamic-pituitary-adrenal (HPA) axis dysfunction and is appropriate for the identification of psychiatric conditions such as depression and anxiety-related disorders (Heuser et al., 1994). The implementation of DEX/CRH challenge, first utilized by von Bardeleben and Holsboer (1989), highlights the altered feedback mechanisms related to said pathologies. In patients suffering from depression or melancholia, stimulus with exogenous CRH results in an escape from dexamethasone suppression that is normally observed in healthy individuals. Findings in murine behavioral-based models echo those of the human clinical setting, as both Roman low avoidance rats (Steimer et al. 2007) and high anxiety-related behavior rats (Keck et al. 2002) exhibit increased corticosterone and ACTH response following DEX/CRH challenge. Given this, it seems appropriate to utilize this test on the bovine temperament model to identify any parallels with results observed in other endophenotype models as well as with observations of patients with disorders rooted in HPA dysfunction.

The objective of the following experiment was to characterize the pituitary and adrenal hormone profiles observed in excitable and calm cattle, following administration of the DEX/CRH challenge.

Materials and Methods

Preliminary Dexamethasone Dose Trial

A total of 7 bulls, approximately 2 1/2 years of age, were utilized in an initial pilot study to investigate the temporal dynamics of pituitary and adrenal suppression following dexamethasone treatment. All of the following procedures have been reviewed and approved by the Texas A&M University IAACUC (AUP 2006-134). Animals were randomly assigned to one of three treatment groups: Controls received injection of sterile saline (n = 3), low DEX received injection of dexamethasone at 0.02 mg/kg BW (n = 2), and high DEX received injection of dexamethasone at 0.04 mg/kg BW (n = 2). Each animal was fitted with an indwelling jugular catheter, as described by Curley et al. (2008), 18 h prior to dexamethasone treatment. At the time of catheterization one 10-ml blood sample was collected, via the jugular catheter (see Figure 5 for visual representation of the sampling regimen). During the period of catheterization and pre-dexamethasone blood sampling, as well as the period of intensive blood sampling, the bulls were penned together and given free choice access to water and Coastal bermudagrass hay. Four hours prior to dexamethasone treatment the bulls were removed from the pen, haltered, and tied within segments of a chute system. Blood sampling at 0.5-h intervals, via the jugular catheters, began 3 h prior to

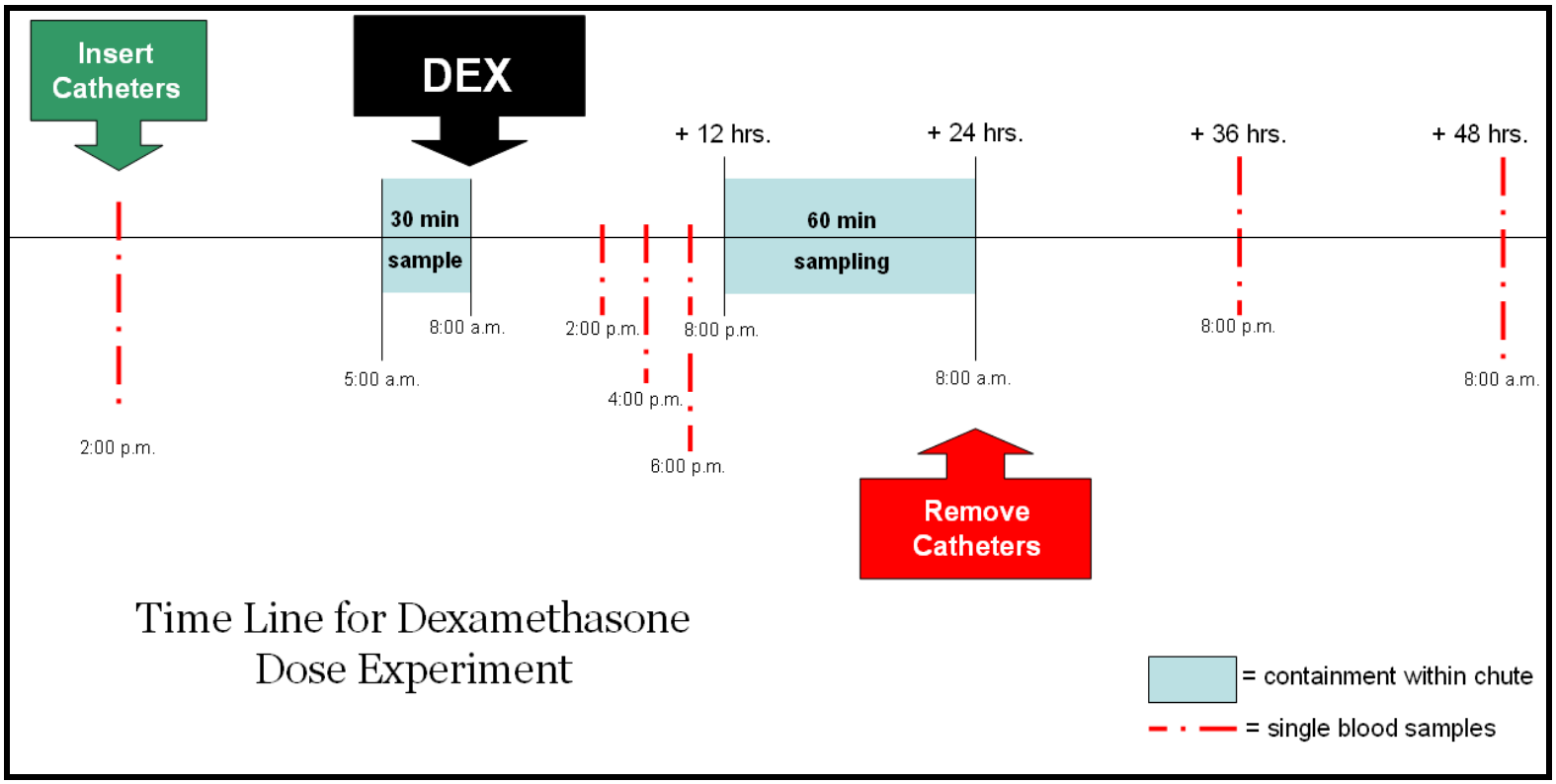

Figure 5. Schematic of the serial blood sampling schedule used in the preliminary testing of dexamethasone dosages.

dexamethasone treatment. Following the administration of dexamethasone (0, 0.02, 0.04 mg/kg BW in accordance with treatment group allocations) the bulls were untied and returned to the pen. At 6, 8, and 10 h post-dexamethasone treatment the bulls were taken through the chute system in order to obtain a single blood sample, and then returned to the pen. Eleven hours post dexamethasone treatment the bulls were removed from the pen, and again tied within segments of a chute system. Blood sampling at 1-h intervals, via the jugular catheters, commenced 12 h post dexamethasone treatment and continued through 24 h post-dexamethasone treatment. At this point the catheters were removed and the animals returned to the pen. At 36 and 48 h post-dexamethasone treatment the bulls were run through the chute system in order to obtain single blood samples via coccygeal venipuncture. Following a 28-d resting period the bulls were again randomly assigned to a treatment group other than the one the individuals were previously in, and the entire experiment was repeated yielding n = 6 bulls in the control group and n = 4 bulls in each of the low and high DEX treatment groups.

DEX/CRH Challenge

A total of 10 crossbreed heifers, approximately 1 year of age, were utilized to compare the pituitary and adrenal response to pharmacological stimulus administered 24 h post dexamethasone treatment, in calm and temperamental cattle. All of the following procedures have been reviewed and approved by the Texas A&M University IAACUC (AUP 2006-194). Cattle temperament was determined via exit velocity so that the calm and temperamental groups consist of the 5 slowest and 5 fastest heifers from a particular

calf crop. Exit velocity (Burrow et al., 1988) is the rate at which the animals exit a squeeze chute and traverse a fixed distance (1.83 m). Throughout the duration of the experiment, except for the time periods of blood sampling, the heifers were penned together and given free choice access to water and Coastal bermudagrass hay. Each animal was fitted with an indwelling jugular catheter, as described by Curley et al. (2008), 2 h prior to the initiation of serial blood sampling.

Six-and-one-half h prior to dexamethasone treatment the heifers were removed from the pen, haltered, and tied within segments of a chute system. Blood sampling (Figure 6) at 0.5-h intervals, via the jugular catheters, began 6 h prior to dexamethasone treatment. Following the administration of dexamethasone (0.02 mg/kg BW) the heifers were untied and returned to the pen. Eighteen and one half hours post dexamethasone treatment the heifers were again removed from the pen, and tied within segments of a chute system. Blood sampling at 0.5-h intervals, via the jugular catheters, resumed 18 h post dexamethasone treatment until 24 h post dexamethasone. A CRH challenge (0.1 μg/kg BW) was then administered via the jugular catheters at 24 h post dexamethasone. Following the CRH treatment, blood collections continued at 5-minute intervals until 25 h post dexamethasone, and then collection intervals increased to 15 minutes. This regimen was maintained for 2 h when the intervals increased again, to 30 minutes, and remained constant until the final samples were collected at 30 h post dexamethasone treatment. At this point the catheters were removed and the animals returned to the pen.

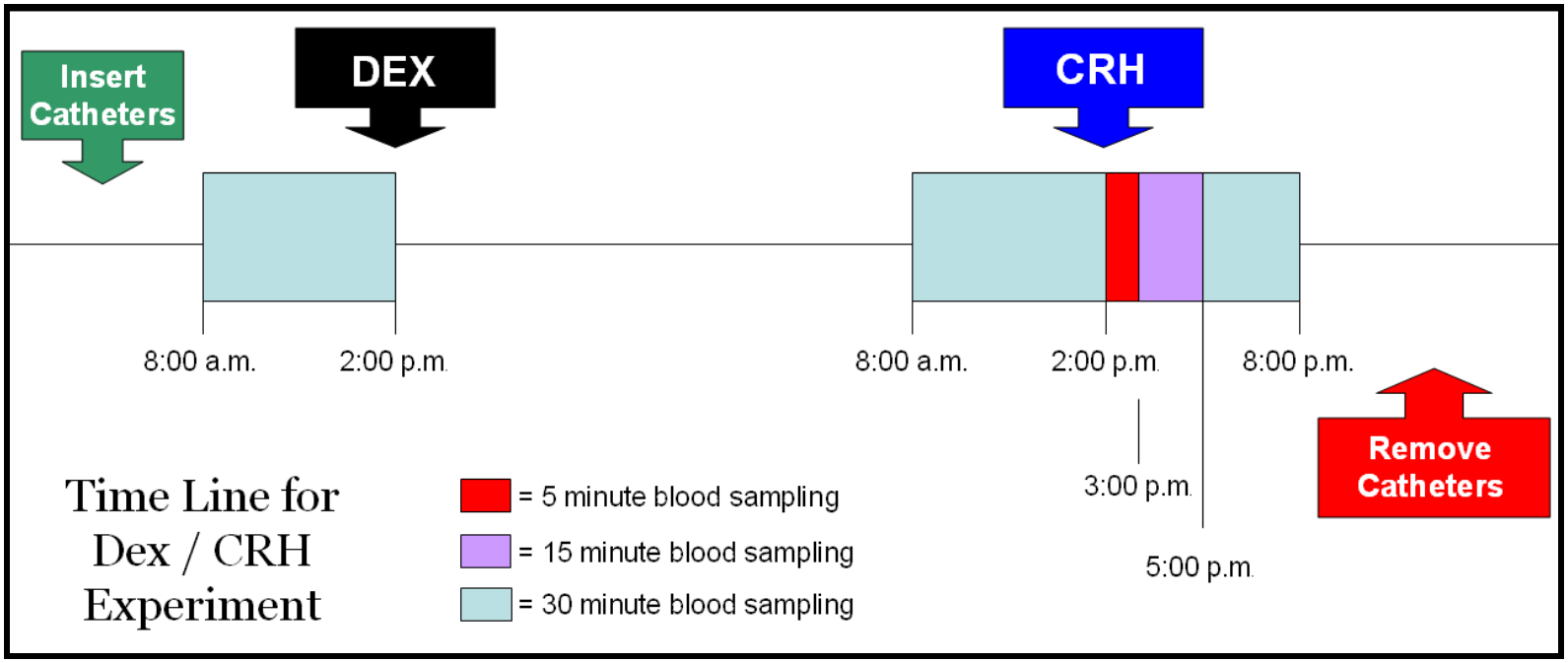

Figure 6. Schematic of the serial blood sampling regimen used in the DEX/CRH challenge.

Data Collection

All blood samples were collected into 10-ml vacutainers containing EDTA and stored on ice. Samples were immediately placed in an ice bath and centrifuged at 4 °C within 3 h of collection. Plasma samples were frozen and stored at −20 °C until cortisol concentrations were analyzed via radioimmunoassay

Cortisol concentrations were determined from duplicate samples using a single antibody RIA procedure (Carroll et al., 2007) and utilized: rabbit anti-cortisol antiserum (Pantex, Div. of Bio-Analysis Inc., Santa Monica, CA, Cat. #P44) diluted 1:2500; standards made by serial dilution (8000 pg/100 μL to 3.9 pg/100 μL) of 4-pregnen-11β,17,21-triol-3,20-dione (Steraloids Inc., Newport, RI, Cat. #Q3880-000); and radio-labeled cortisol: 3H-Hydrocortisone (1,2-3H, NEN, Boston, MA, Cat. #NET-185). Counts per minute (cpm) were obtained from a liquid scintillation spectrophotometric beta-counter (Beckman Coulter LS 6500) and unknown cortisol concentrations were calculated using Assay Zap software (Biosoft, Cambridge, UK). Cortisol antiserum cross-reactivity was: corticosterone, 60%; deoxycorticosterone, 48%; progesterone, 0.01%; and estradiol, 0.01%, (determined by Pantex). Interassay and intraassay CV were 8.7% and 12.1%, respectively.

Statistical Analysis

Repeated measures analysis, specific for mixed models, was conducted to determine the effects of time, treatment, and time x treatment on hormone concentrations throughout the duration of blood sampling. Within the statistical model a random effect

from individual animals is accounted for. Additionally, the correlation between repeated samples, within each animal, is modeled using a heterogeneous autoregressive covariance structure. The GLM procedure of SAS (SAS Inst., Inc., Cary, NC) was utilized for ANOVA of treatment group effects on concentrations of cortisol at certain key time points.

Results and Discussion

Preliminary Dexamethasone Dose Trial

Prior to the administration of DEX, concentrations of cortisol were the highest observed throughout the duration of the trial and there was no difference ($P = 0.95$) between the control and either of the treatment groups. Over the ensuing 3 h, cortisol concentrations steadily declined in all bulls and at the time of DEX injection there was again no difference ($P = 0.59$) amongst either of the doses tested. While both the acclimation to the experimental surroundings and the recovery from handling stress would certainly explain the decline in cortisol during this time, it is also important to note the source of these bulls. As the goal of this preliminary trial was only to assess the efficacy of DEX dosage, these animals were selected so that temperament would not impact this portion of the study. Specifically, as these particular bulls were primarily used for estrus detection and had been on the ranch for over 2 years, they were quite acclimated to our facilities and handlers. It is likely this familiarity was the cause of the pre-DEX concentrations of cortisol being uncommonly low. Cortisol profiles for the entirety of sampling are depicted in Figure 7.

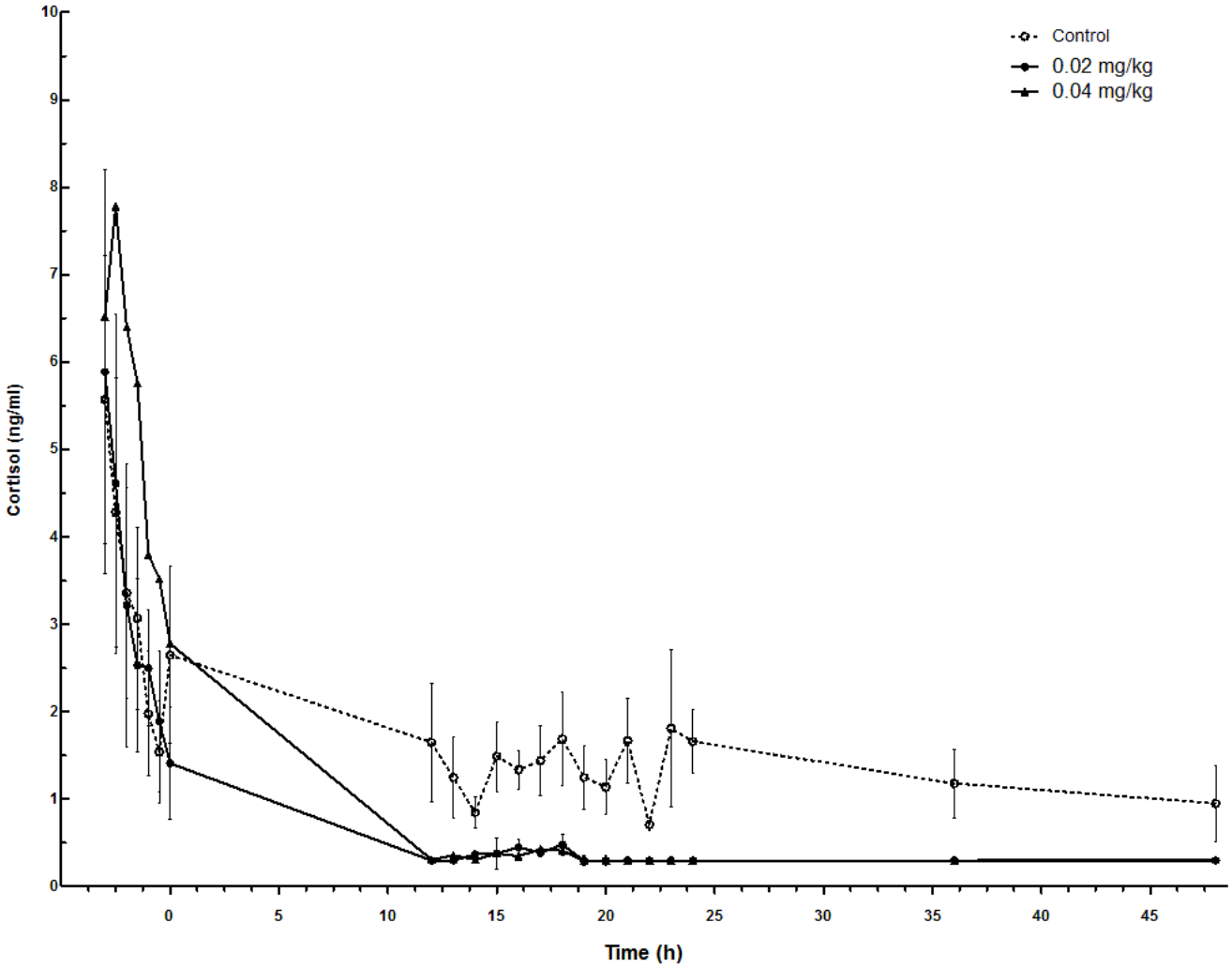

Figure 7. Mean plasma concentrations of cortisol before and after administration of dexamethasone (DEX) in bulls given a dose of 0.02 mg/kg (closed circle), 0.04 mg/kg (closed triangle), and saline (open circle). DEX administered at time 0.

When sampling resumed 12 h post-DEX, both of the treated groups were lower ($P < 0.06$) than that of the control group, yet did not differ with dosage. Dexamethasone treatment had effectively suppressed the HPA function in these bulls, rendering the circulating cortisol concentrations negligible. The handling associated with recommencement of blood sampling did stimulate a slight increase in adrenal activity; however, cortisol concentrations in both of the DEX treated groups never reached 1.0 ng/mL. Concentrations of cortisol 24 h post-DEX conveyed a similar story, in that those treated with DEX were markedly lower ($P < 0.005$) than the control bulls, yet not different across dose range. Continued suppression after 24 h would agree with observations of cortisol concentrations in dairy cows following treatment with 0.1 mg/kg of dexamethasone-21-isonicotinate, as adrenal function in those animals returned to normal between 24 and 72 h (Toutain et al., 1982). By 48 h post-DEX, cortisol was numerically lower in both the treated groups; however, high variation within the control group resulted in no statistical significance ($P = 0.26$) in these concentrations. As both dosages of DEX effectively suppressed adrenal function in the bulls tested and there was no appreciable difference related to the specific dosage, we opted to utilize the lesser dose, 0.02 mg/kg BW, in the combined DEX/CRH challenge.

DEX/CRH Challenge

While conducting the radioimmunoassay for cortisol in the plasma collected during this trial it quickly became apparent that the majority of these samples contained concentrations well below the sensitivity of our assay, which is estimated at ~100

pg/mL. Although this also occurred when processing the samples from the preliminary dose trial, only about 15% of those were undetectable by our assay system. In contrast, over 70% of the samples assayed from the combined DEX/CRH challenge were outside the range of our assay. Given the questionable biological relevance of cortisol at these concentrations, I was advised to discontinue further assay of these samples in order to preserve resources. Therefore, no meaningful data were generated from this trial.

The most perplexing aspect is that even with the DEX-treated bulls, used during the preliminary trial, there was evidence of adrenal response to the agitation of being situated within the chute system 12 h after DEX. As these animals were much easier to handle than the excitable heifers used in the DEX/CRH trial, it seems curious that similar handling did not produce a measureable adrenal response. One possibility is that a mistake was made while calculating the individual dosages of DEX administered to the heifers. However, all calculations were checked multiple times by two technicians. Additionally, DEX dosages 5X larger than those employed herein still permitted a measurable adrenal response to exogenous ACTH administered 24 h post DEX to dairy cows (Toutain et al., 1982).

CHAPTER VI

CONCLUSIONS

The inherent complexities of biological systems that incorporate genotype-environment interactions are clearly evident in the bovine temperament model. Links between fearfulness, psychological disorders, and altered HPA function have been well established in humans, lab animals, and domestic agricultural species. Our working hypothesis maintains that the behavioral excitability characterized by cattle temperament measures is associated with dysfunction of the endocrine stress axis. Whether changes in HPA activity are the underlying cause or just manifestations of an excitable temperament remains to be determined—although important, it was not a focus of the studies that constitute this body of work. The goal of these experiments was to further characterize the endophenotypes associated with this behavior-stress paradigm. Identification of these "internal" phenotypes (Gottesman and Shields, 1973) helps to construct a composite of the measurable biological markers associated with complex pathophysiologies. A greater understanding of the physiologic mechanisms associated with cattle temperament would not only be beneficial to the beef industry, but may also lead to an animal partial-model relevant to stress-related psychopathologies in humans.

A blunted pituitary response to CRH stimulation has been identified as a characteristic of cattle with an excitable temperament. Since VP works in concert with CRH to stimulate pituitary activity, we set out to determine if temperament impacted the function of VP as an ACTH secretagogue; the details of which are described in Chapter

III. By using exit velocity as a temperament selection criterion, steers with an excitable or calm temperament were identified and subjected to serial blood sampling for a period of 12 h. Midway through these collections, all animals received a bolus of exogenous VP. Concentrations of cortisol in excitable steers during the pre-challenge period revealed an increased initial adrenal reactivity to interactions with humans. Subsequent acclimation to the experimental surroundings yielded greater baseline cortisol concentrations in cattle with an excitable temperament; both of these observations adhere to an existing endophenotype of hypercortisolism (Curley et al., 2006; Burdick et al., 2011a). Pituitary stimulation with VP resulted in a greater ACTH output from the excitable steers compared to their calm counterparts. Interestingly, we observed just the opposite when cattle of calm temperament exhibited a greater pituitary response to exogenous bCRH (Curley et al., 2008). This particular dichotomy may suggest a fundamental difference at the corticotroph level; however, the specific mechanisms of which remain unclear. These differences in secretagogue function may be linked to CRH and VP receptor dynamics, alterations in feedback inhibition by glucocorticoids, or a combination thereof. The addition of an exaggerated pituitary response to VP as another endophenotype associated with an excitable temperament warrants specific focus on corticotroph function in further research.

Various stereotypes associating specific breeds with a preferable temperament exist within the beef industry. Of particular significance are the multitude of breed comparisons that document the great distinction between the temperament of *Bos taurus* and *Bos indicus* breeds. A commonly-held belief is that Brahman cattle are more

excitable and become more stressed when compared to English breeds such as Angus (Grandin, 1980). We utilized this supposition to identify if breed type impacted the dynamic between temperament and HPA function; the resultant experiment is the focus of Chapter IV. Again, cattle temperament was identified via EV measures and steers of an excitable or calm temperament were selected from Brahman and Angus calf crops. Function of the HPA axis was assessed via exogenous CRH challenge as had been previous utilized with Brahman heifers (Curley et al., 2008). When separately considered from that of the Brahman steers, the data from the Angus steers parallel our previous observations from the Brahman heifers: a blunted pituitary response in the animals of poor temperament. Not only does this strengthen the validity of an existing endophenotype, but it also quells any idea that those prior observations were relevant only to Brahman cattle (a breed well-known for poor temperament) and suggests that this is likely a characteristic applicable across breed type.

The specific endophenotypes which have been identified or reinforced through the research described herein suggest that there are indeed aspects of HPA dysfunction associated with bovine temperament. Moreover, direct parallels to human stress-related psychopathologies, e.g., the enhanced ACTH response to VP (Dinan et al., 2004) and the diminished response to pituitary stimulation with CRH (Gold and Chrousous, 1985) observed in depressed patients, support the relevance of our observations in the bovine species. As such, it would be useful to seek out other endophenotypes associated with cattle temperament. Genomic comparisons via QTL analysis of temperament traits in beef and dairy cattle suggest dopamine metabolism (Hiendleder et al., 2003) and the

dopamine receptor (Gutiérrez-Gil et al., 2008; Glenske et al., 2011) as possible avenues for future research. Additionally, it would be advantageous to further explore the nuances of the enophenotypes we have already established, specifically the dynamics of pituitary stimulation and regulation with regard to an excitable temperament.

LITERATURE CITED

Agado, B. A., K. O. Curley Jr., J. G. Lyons, D. A. Neuendorff, A. W. Lewis, T. D. A. Forbes, T. H. Welsh, Jr., and R. D. Randel. 2009. Serum concentrations of cortisol induced by exogenous adrenocorticotropic hormone (ACTH) are not predictive of residual feed intake (RFI) in Brahman cattle. J. Anim. Sci. 87(E-Suppl. 3):41. Abstract.

Aguilera, G. 1994. Regulation of pituitary ACTH secretion during chronic stress. Front. Neuroendocrin. 15:321-350.

Aguilera, G., and C. Rabadan-Diehl. 2000. Vasopressinergic regulation of the hypothalamic-pituitary-adrenal axis: implications for stress adaptation. Regul. Peptides 96:23-29.

Andrade, O., A. Orihuela, J. Solano, and G. S. Galina. 2001. Some effects of repeated handling and the use of a mask on stress responses in zebu cattle during restraint. Appl. Anim. Behav. Sci. 71:175-181.

Antoni, F. A. 1987. Receptors mediating the CRH effects of vasopressin and oxytocin. Ann. NY Acad. Sci. 512:195-204.

Antoni, F. A. 1993. Vasopressinergic control of pituitary adrenocorticotropin secretion comes of age. Front. Neuroendocrin.14:76-122.

Arriza, J. L., R. B. Simerly, L. W. Swanson, and R. M. Evans. 1988. The neuronal mineralocorticoid receptor as a mediator of glucocorticoid response. Neuron 1:887–900.

Aubry, J. M., V. Bartanusz, P. Driscoll, P. Schulz, T. Steimer, and J. Z. Kiss. 1995. Corticotropin-releasing factor and vasopressin mRNA levels in Roman high- and low-avoidance rats: response to open field exposure. Neuroendocrinology 61:89–97.

Axelrod, J., and T. D. Reisine. 1984. Stress hormones: Their interaction and regulation. Sci. 224:452-459.

Bearden, C. E., and N. B. Freimer. 2006. Endophenotypes for psychiatric disorders: ready for primetime? Trends Genet. 22:306-313.

Behrends, S. M., R. K. Miller, F. M. Rouquette, Jr., R. D. Randel, B. G. Warrington, T. D. A. Forbes, T. H. Welsh, H. Lippke, J. M. Behrends, G. E. Carstens, and J. W. Holloway. 2009. Relationship of temperament, growth, carcass characteristics and tenderness in beef steers. Meat Sci. 81:433-438.

Bell, P. H., K. S. Howard, R. G. Shepherd, B. M. Finn, and J. H. Meisenhelder. 1956. Studies with corticotrophs. II. Pepsin degradation of b-corticotropin. J. Am. Chem. Soc. 78:5059-5066.

Bignami, G., 1965. Selection for high rates and low rates of avoidance conditioning in the rat. Anim. Behav.13:221–227.

Blecha, F., S. L. Boyles, and J. G. Riley. 1984. Shipping suppresses lymphocyte blastogenic responses in Angus and Brahman x Angus feeder calves. J. Anim. Sci. 59:576-583.

Boissy, A. 1995. Fear and fearfulness in animals. Q. Rev. Biol. 70:165-191.

Boissy, A., and M. F. Bouissou. 1988. Effect of early handling on heifers subsequent reactivity to humans and to unfamiliar situations. Appl. Anim. Behav. Sci. 20:259-273.

Boivin, X., P. Le Neindre, J. P. Garel, and J. M. Chupin. 1994. Influence of breed and rearing management on cattle reactions during human handling. Appl. Anim. Behav. Sci. 39:115-122.

Bradbury, M. J., S. F. Akana, C. S. Cascio, N. Levin, L. Jacobson, and M. F. Dallman. 1991. Regulation of basal ACTH secretion by corticosterone is mediated by both type I (MR) and type II (GR) receptors in the rat brain. J. Steroid Biochem. Molec. Biol. 40:133-142.

Bristow, D. J., and D. S. Holmes. 2007. Cortisol levels and anxiety-related behaviors in cattle. Physiol. Behav. 90:626-628.

Burdick, N. C., B. Agado, J. C. White, K. J. Matheney, D. A. Neuendorff, D. G. Riley, R. C. Vann, T. H. Welsh, Jr., and R. D. Randel. 2011a. Technical note: Evolution of exit velocity in suckling Brahman calves. J. Anim. Sci. 89:233-236.

Burdick, N. C., J. A. Carroll, L. E. Hulbert, J. W. Dailey, M. A. Ballou, R. D. Randel, S. T. Willard, R. C. Vann, and T. H. Welsh, Jr. 2011b. Temperament influences endotoxin-induced changes in rectal temperature, sickness behavior, and plasma epinephrine concentrations in bulls. Innate Immun. 17:355-364.

Burdick, N. C., J. A. Carroll, L. E. Hulbert, J. W. Dailey, S. T. Willard, R. C. Vann, T. H. Welsh, Jr., and R. D. Randel. 2010. Relationships between temperament and transportation with rectal temperature and serum concentrations of cortisol and epinephrine in bulls. Livest. Sci. 129:166–172.

Burrow, H. M. 1997. Measurements of temperament and their relationships with performance traits of beef cattle. Anim. Breeding Abstr. 65:477-495.

Burrow, H. M. 2001. Variances and covariances between productive and adaptive traits and temperament in a composite breed of tropical cattle. Livest. Prod. Sci. 70:213-233.

Burrow, H. M., and N. J. Corbet. 2000. Genetic and environmental factors affecting temperament of zebu and zebu-derived beef cattle grazed at pasture in the tropics. Aust. J. Agric. Res. 51:155-162.

Burrow, H. M., and R. D. Dillon. 1997. Relationships between temperament and growth in a feedlot and commercial carcass traits of *Bos indicus* crossbreds. Aust. J. Exp. Agr. 37:407-411.

Burrow, H. M., and K. C. Prayaga. 2004. Correlated responses in productive and adaptive traits and temperament following selection for growth and heat resistance in tropical beef cattle. Livest. Prod. Sci. 86:143-161.

Burrow, H. M., G. W. Seifert, and N. J. Corbet. 1988. A new technique for measuring temperament in cattle. Proc. Aust. Soc. Anim. Prod. 17:154-157.

Cafe, L. M., D. L. Robinson, D. M. Ferguson, B. L. McIntyre, G. H. Geesink, and P. L. Greenwood. 2011a. Cattle temperament: persistence of assessments and associations with productivity, efficiency, carcass and meat quality traits. J. Anim. Sci. 89:1452-1464.

Cafe, L. M., D. L. Robinson, D. M. Ferguson, B. L. McIntyre, G. H. Geesink, and P. L. Greenwood. 2011b. Temperament and hypothalamic-pituitary-adrenal axis function are related and combine to affect growth, efficiency, carcass, and meat quality traits in Brahman steers. Domest. Anim. Endocrinol. 40:230-240.

Cameron, E. H. D., T. Jones, D. Jones, A. B. M. Anderson, and K. Griffiths. 1969. Further studies on the relationship between C19- and C21-steroid synthesis in the human adrenal gland. J. Endocrinol. 45: 215-230.

Cannon, W. B. 1932. The Wisdom of the Body. W.W. Norton, New York, NY.

Carroll, J. A., N. H. McArthur, and T. H. Welsh, Jr. 2007. In vitro and in vivo temporal aspects of ACTH secretion: stimulatory actions of corticotropin-releasing hormone and vasopressin in cattle. J. Vet. Med. A Physiol. Pathol. Clin. Med. 54:7-14.

Childs, G. V. 1992. Structure-function correlates in the corticotropes of the anterior pituitary. Front. Neuroendocrinol. 13:271-317.

Cooke, R. F., J. D. Arthington, D. B. Araujo, and G. C. Lamb. 2009. Effects of acclimation to human interaction on performance, temperament, physiological responses, and pregnancy rates of Brahman-crossbred cows. J. Anim. Sci. 87:4125-4132.

Curley, K. O., Jr., J. C. Paschal, T. H. Welsh, Jr., and R. D. Randel. 2006. Technical Note: Exit velocity as a measure of cattle temperament is repeatable and associated with serum concentration of cortisol in Brahman bulls. J. Anim. Sci. 84:3100-3103.

Curley, K. O., Jr., D. A. Neuendorff, A. W. Lewis, J. J. Cleere, T. H. Welsh, Jr., and R. D. Randel. 2008. Functional characteristics of the bovine hypothalamic–pituitary–adrenal axis vary with temperament. Horm. Behav. 53:20-27.

Curley, K. O., Jr., D. A. Neuendorff, A. W. Lewis, F. M. Rouquette, Jr., T. H. Welsh, Jr., and R. D. Randel. 2010. Effectiveness of vasopressin as an ACTH secretagogue in cattle differs with temperament. Physiol. Behav.101: 699-704.

Dallman, M. F. 1993. Stress update: Adaptation of the hypothalamic-pituitary-adrenal axis to chronic stress. Trends Endocrinol. Metab. 4:62-69.

Dallman, M. F., S. F. Akana, K. A. Scribner, M. J. Bradbury, C. D. Walker, A. M. Strack, and C. S. Cascio. 1992. Stress, feedback and facilitation in the hypothalamo–pituitary–adrenal axis. J. Neuroendocrinol. 4:517–526.

Dallman, M. F., and M. T. Jones. 1973. Corticosteroid feedback control of ACTH secretion: effect of stress-induced corticosterone secretion on subsequent stress responses in the rat. Endocrinol. 92:1367–1375.

Datson, N. A., J. van der Perk, E. R. de Kloet, and E. Vreugdenhil. 2001. Identification of corticosteroid-responsive genes in rat hippocampus using serial analysis of gene expression. Eur. J. Neurosci. 14:675–689.

de Kloet, E. R., E. Vreugdenhil, M. S. Oitzl, and M. Joels. 1998. Brain corticosteroid receptor balance in health and disease. Endo. Rev. 19:269-301.

de Kloet, E.R., G. Wallach, and B. S. McEwen. 1975. Differences in corticosterone and dexamethasone binding to rat brain and pituitary. Endocrinology 96:598–609.

del Campo, M., G. Brito, J. Soares de Lima, P. Hernandez, and F. Montossi. 2010. Finishing diet, temperament and lairage time effects on carcass and meat quality traits in steers. Meat Sci. 86:908-914.

Dhabhar, F. S., B. S. McEwen, and R. L. Spencer. 1997. Adaptation to prolonged or repeated stress – Comparison between rat strains showing intrinsic differences in reactivity to acute stress. Neuroendocrinology. 65:360-368.

Dickson, D. P., G. R. Barr, L. P. Johnson, and D. A. Wieckert. 1970. Social dominance and temperament of Holstein cows. J. Dairy Sci. 53:904-907.

Dinan, T. G., S. O'Brein, E. Lavelle, and L. V. Scott. 2004. Further neuroendocrine evidence of enhanced vasopressin V3 receptor responses in melancholic depression. Psychol. Med. 34:169-172.

Dinan, T. G., and L. V. Scott. 2005. Anatomy of melancholia: focus on hypothalamic-pituitary-adrenal axis overactivity and the role of vasopressin. J. Anat. 207:259-264.

Espiner, E. A., C. A. Jensen, and D. S. Hart. 1972. Dynamics of adrenal response to sustained local ACTH infusions in conscious sheep. Am. J. Physiol. 222:570-577.

Evans, M. J., N. E. Kitson, J. H. Livesey, and R. A. Donald. 1993. The effect of cortisol on the secretion of ACTH by anterior-pituitary-cells of the horse in culture. J. Endocrinol. 137:403-412.

Fell, L. R., I. G. Colditz, K. H. Walker, and D. L. Watson. 1999. Associations between temperament, performance and immune function in cattle entering a commercial feedlot. Aust. J. Exp. Agr. 39:795-802.

Fordyce, G., R. M. Dodt, and J. R. Wythes. 1988a. Cattle temperaments in extensive beef herds in northern Queensland. 1: Factors affecting temperament. Aust. J. Exp. Agr. 28:683-687.

Fordyce, G., M. E. Goddard, and G. W. Seifert. 1982. The measurement of temperament in cattle and the effect of experience and genotype. P. Aust. Soc. Anim. Prod. 14:329-332.

Fordyce, G., C. J Howitt, R. G. Holroyd, P. K. O'Rourke, and K. W. Entwistle. 1996. The performance of Brahman-Shorthorn and Sahiwal-Shorthorn beef cattle in the dry tropics of northern Queensland. 5: Scrotal circumference, temperament, ectoparasite resistance, and the genetics of growth and other traits in bulls. Aust. J. Exp. Agr. 36:9-17.

Fordyce, G., J. R. Wythes, W. R. Shorthose, D. W. Underwood, and R. K. Shepherd. 1988b. Cattle temperaments in extensive beef herds in northern Queensland. 2: Effect of temperament on carcass and meat quality. Aust. J. Exp. Agr. 28:689 693.

Gentsch, C. M., M. Lichsteiner, P. Driscoll, and H. Feer. 1982. Differential hormone and physiological responses to stress in Roman high and low-avoidance rats. Physiol. Behav. 28:259-263.

Gibbons, J. M., A. B. Lawrence, and M. J. Haskell. 2011. Consistency of flight speed and response to restraint in a crush in dairy cattle. Appl. Anim. Behav. Sci. 131:15-20.

Gillies, G. E., E. A. Linton, and P. J. Lowry. 1982. Corticotropin releasing activity of the new CRF is potentiated several times by vasopressin. Nature 299:355-357.

Glenske, K., E. M. Prinzenberg, H. Brandt, M. Gauly, and G. Erhardt. 2011. A chromosome-wide QTL study on BTA29 affecting temperament traits in German Angus beef cattle and mapping of DRD4. Anim. 5:195-197.

Gold, P. W., and G. P. Chrousos. 1985. Clinical studies with corticotropin releasing factor: Implications for diagnosis and pathophysiology of depression, Cushing's disease, and adrenal insufficiency. Psychoneuroendocrinol. 10:401-419.

Gottesman, I. I., and T. D. Gould. 2003. The endophenotype concept in psychiatry: etymology and strategic intentions. Am. J. Psychiat. 160:636-645.

Gottesman, I. I., and J. Shields. 1973. Genetic theorizing and schizophrenia. Brit. J. Psychiat. 122:15-30.

Gould, T. D., and I. I. Gottesman. 2006. Psychiatric endophenotypes and the development of valid animal models. Genes Brain Behav. 5:113-119.

Grandin, T. 1980. Livestock behavior as related to handling facilities design. Int. J. Stud. Anim. Prob. 1:33-42.

Grandin, T. 1993. Behavioral agitation during handling of cattle is persistent over time. Appl. Anim. Behav. Sci. 36:1-9.

Grandin, T. 1997. Assessment of stress during handling and transport. J. Anim. Sci. 75:249-257.

Grammatopoulos, D. K., and G. P. Chrousos. 2002. Functional characteristics of CRH receptors and potential clinical applications of CRH-receptor antagonists. Trends Endocrinol. Metab. 13:436-444.

Griffiths, K., J. K. Grant, and T. Symington. 1963. A biochemical investigation of the functional zonation of the adrenal cortex in man. J. Clin. Endocrinol. Metab. 23:776-785.

Gutiérrez-Gil, B., N. Ball, D. Burton, M. Haskell, J. L. Williams, and P. Wiener. 2008. Identification of quantitative trait loci affecting cattle temperament. J. Hered. 99:629-638.

Hafez, E. S. E., and D. R. Lindsay. 1965. Behavioral responses in farm animals and their relevance to research techiques. Animal Breeding Abstracts 33:1-16.

Hall, N. L., D. S. Buchanan, V. L. Anderson, B. R. Ilse, K. R. Carlin, and E. P. Berg. 2011. Working chute behavior of feedlot cattle can be an indication of cattle temperament and beef carcass composition and quality. Meat Sci. 89:52-57.

Hammond, A. C., T. A. Olson, C. C. Chase Jr, E. J. Bowers, R. D. Randel, C. N. Murphy, D. W. Vogt, and A. Tewolde. 1996. Heat tolerance in two tropically adapted *Bos taurus* breeds, Senepol and Romosinuano, compared with Brahman, Angus, and Hereford cattle in Florida. J. Anim. Sci. 74:295-303.

Harris, G. 1948. Neural control of the pituitary gland. Physiol. Rev. 28:139-179.

Hasler, G., W. C. Drevets, H. K. Manji, and D. S. Charney. 2004. Discovering endophenotypes for major depression. Neuropshychopharmacol. 29:1765-1781.

Hauger, R. L., M. A. Millan, M. Lorang, J. P. Harwood, and G. Aguilera. 1988. Corticotropin- releasing factor receptors and pituitary adrenal responses during immobilization stress. Endocrinol.123:396-405.

Hearnshaw, H., R. Barlow, and G. Want. 1979. Development of a “temperament” or “handling difficulty” score for cattle. Aust. Assoc. Anim. Breeding Genet. 1:164-166.

Hearnshaw, H., and C. A. Morris. 1984. Genetic and environmental effects on a temperament score in beef cattle. Aust. J. Agric. Res. 35:723-733.

Hechter, O. 1949. Corticosteroid release from the isolated adrenal gland. Fed. Proc. 8:70-71.

Hench, P. S., E. C. Kendall, C. H. Slocumb, and H. F. Polley. 1949. The effect of a hormone of the adrenal cortex (17-hydroxy-11-dehydrocorticosterone: compound E) and of pituitary adrenocorticotropic hormone on rheumatoid arthritis. Proc. Mayo Clinic 24:181-197.

Henricks, D. M., J. W. Cooper, J. C. Spitzer, and L. W. Grimes. 1984. Sex differences in plasma cortisol and growth in the bovine. J. Anim. Sci. 59:376-383.

Heuser, I., A. Yassouridis, and F. Holsboer. 1994. The combined dexamethasone/CRH test: a refined laboratory test for psychiatric disorders. J. Psychiatr. Res. 28:341–356.

Hiendleder S., H. Thomsen, N. Reinsch, J. Bennewitz, B. Leyhe-Horn, C. Looft, N. Xu, I. Medjugorac, I. Russ, C. Kuhn, G. A. Brockmann, J. Blümel, B. Brenig, F. Reinhardt, R. Reents, G. Averdunk, M. Schwerin, M. Förster, E. Kalm, and G. Erhardt. 2003. Mapping of QTL for body conformation and behavior in cattle. J. Hered. 94:496-506.

Hogg, S. 1996. A review of the validity and variability of the elevated plus-maze as an animal model of anxiety. Pharmacol. Biochem. Behav. 54:21–30.

Hoppe, S., H. R. Brandt, S. Konig, G. Erhardt, and M. Gauly. 2010. Temperament traits of beef calves measured under field conditions and their relationships to performance. J. Anim. Sci. 88:1982-1989.

Hulbert, L. E., J. A. Carroll, N. C. Burdick, R. D. Randel, M. S. Brown, and M. A. Ballou. 2011. Innate immune responses of temperamental and calm cattle after transportation. Vet. Immunol. Immunop. 143:66-74.

Ingle, D. J. and E. C. Kendall. 1937. Atrophy of the adrenal cortex of the rat produced by administration of large amounts of cortin. Science 86:245.

Janssens, C. J. J. G., F. A. Helmond, and V. M. Wiegant. 1995. Chronic stress and pituitary-adrenocortical responses to corticotrophin-releasing hormone and vasopressin in female pigs. Eur. J. Endocrinol. 132:479-486.

Jones, R. B., A. D. Mills, J. M. Faure, and J. B. Williams. 1994. Restraint, fear, and distress in Japanese quail genetically selected for long or short tonic immobility reactions. Physiol. Behav. 56:529–534.

Kaplan, N., and F. C. Bartter. 1962. The effect of ACTH, renin, angiotensin II, and various precursors on biosynthesis of aldosterone by adrenal slices. J. Clin. Invest. 41:715-724.

Keck, M. E., A. Wigger, T. Welt, M. B. Müller, A. Gesing, J. M. H. M. Reul, F. Holsboer, R. Landgraf, and I. D. Neumann. 2002. Vasopressin mediates the response of the combined dexamethasone/CRH test in hyper-anxious rats: implications for the pathogenesis of affective disorders. Neuropsychopharmacol. 26:94–105.

Keller-Wood, M. E., and M. F. Dallman. 1984. Corticosteroid inhibition of ACTH secretion. Endocr. Rev. 5:1-24.

Kendall, E. C. 1949. The chemistry and partial synthesis of adrenal steroids. Ann. NY Acad. Sci. 50:540-547.

Kilgour, R. J., G. J. Melville, and P. L. Greenwood. 2006. Individual differences in the reaction of beef cattle to situations involving social isolation, close proximity of humans, restraint and novelty. Appl. Anim. Behav. Sci. 99:21-40.

King, D. A., C. E. Schuehle Pfeiffer, R. D. Randel, T. H. Welsh, Jr., R. A. Oliphint, B. E. Baird, K. O. Curley, Jr., R. C. Vann, D. S. Hale, and J. W. Savell. 2006. Influence of animal temperament and stress responsiveness on the carcass quality and beef tenderness of feedlot cattle. Meat Sci. 74:546-556.

Kirschbaum, C., B. M. Kudielka, J. Gaab, N. C. Schommer, and D. H. Hellhammer. 1999. Impact of gender, menstrual cycle phase, and oral contraceptives on the activity of the hypothalamus–pituitary–adrenal axis. Psychosom. Med. 61:154–162.

Kitay, J.I. 1961. Sex differences in adrenal cortical secretion in the rat. Endocrinol. 68:818–824.

Knights, M., and G. W. Smith. 2007. Decreased ACTH secretion during prolonged transportation stress is associated with reduced pituitary responsiveness to tropic hormone stimulation in cattle. Domest. Anim. Endocrinol. 33:442-450.

Kudielka, B. M., and C. Kirschbaum. 2005. Sex differences in HPA axis responses to stress: a review. Biologic. Psychol. 69;113-132.

Landgraf, R., and A. Wigger. 2002. High versus low anxiety-related behavior rats: an animal model of extremes in trait anxiety. Behav. Genet. 32:301–314.

Landgraf, R., A. Wigger, F. Holsboer, and I. D. Neumann. 1999. Hyperreactive hypothalamo-pituitary-adrenocortical axis in rats bred for high anxiety-related behavior. J. Neuroendocrinol. 11:405–407.

Liu, J. H., P. Muse, P. Contreras, D. Gibbs, W. Vale, J. Rivier, and S. S. Yen. 1983. Augmentation of ACTH-releasing activity of synthetic corticotropin-releasing factor (CRF) by vasopressin in women. J. Clin. Endocrinol. Metab. 57:1087-1089.

Long, C., B. Katzin, and E. Fry. 1940. The adrenal cortex and carbohydrate metabolism. Endocrinol. 26:309-344.

Mains, R. E., B. A. Eipper, and N. Ling. 1977. Common precursor to corticotropins and endorphins. Proc. Natl. Acad. Sci. U. S. A 74:3014-3018.

Martini, L. and C. Morpurgo. 1955. Neurohumoral control of the release of adrenocorticotropic hormone. Nature 175:1127.

Mason, H. L. 1964. Only the best is good enough. J. Clin. Endocrinol. Metab. 24:1214-1218.

McCann, S. M., and J. R. Brobeck. 1954. Evidence for a role of the supraopticohypophyseal system in the regulation of adrenocorticotrophin secretion. Proc. Soc. Exp. Biol. Med. 87:318-324.

McEwen, B. S., E. R. de Kloet, and W. Rostene. 1986. Adrenal steroid receptors and actions in the nervous system. Physiol. Rev. 66:1121-1188.

Moberg, G. P. 1999. When does stress become distress? Lab Anim. 28:22-26.

Moncek, F., R. Kvetnansky, and D. Jezova. 2001. Differential responses to stress stimuli of Lewis and Fischer rats at the pituitary and adrenocortical level. Endocr. Regul. 35:35-41.

Morris, C. A., N. G. Cullen, R. Kilgour, and K. J. Bremner. 1994. Some genetic factors affecting temperament in *Bos taurus* cattle. N. Z. J. Agric. Res. 37:167-175.

Müller, R., and M. A. G. von Keyserlingk. 2006. Consistency of flight speed and its correlation to productivity and to personality in *Bos taurus* beef cattle. Appl. Anim. Behav. Sci. 99:193-204.

Nishi, M., H. Ogawa, T. Ito, K. I. Matsuda, and M. Kawata. 2001. Dynamic changes in subcellular localization of mineralocorticoid receptor in living cells: in comparison with glucocorticoid receptor using dual-color labeling with green fluorescent protein spectral variants. Mol. Endocrinol. 15:1077–1092.

Nkrumah, J. D., D. H. Crews, Jr., J. A. Basarab, M. A. Price, E. K. Okine, Z. Wang, C. Li, and S. S. Moore. 2007. Genetic and phenotypic relationships of feeding behavior and temperament with performance, feed efficiency, ultrasound, and carcass merit of beef cattle. J. Anim. Sci. 85:2382-2390.

O'Bleness, G. V., Van Vleck, L. D., and Henderson, C. R., 1960. Heritabilities of some type appraisal traits and their genetic and phenotypic correlations with production. J. Dairy Sci. 43:1490-1498.

Pacak, K., M. Palkovits, G. Yadid, R. Kvetnansky, I. J. Kopin, and D. S. Goldstein. 1998. Heterogeneous neurochemical responses to different stressors: a test of Selye's doctrine of nonspecificity. Am. J. Physiol. - Reg. I. 44:R1247-R1255.

Patel, P. D., J. F. Lopez, D. M. Lyons, S. Burke, M. Wallace, and A. F. Schatzberg. 2000. Glucocorticoid and mineralocorticoid receptor mRNA expression in squirrel monkey brain. J. Psychiatr. Res. 34:383–392.

Petherick, J. C., V. J. Doogan, R. G. Holroyd, P. Olsson, and B. K. Venus. 2009a. Quality of handling and holding yard environment, and beef cattle temperament: 1. Relationships with flight speed and fear of humans. Appl. Anim. Behav. Sci. 120:18-27.

Petherick, J. C., V. J. Doogan, B. K. Venus, R. G. Holroyd, and P. Olsson. 2009b. Quality of handling and holding yard environment, and beef cattle temperament: 2. Consequences for stress and productivity. Appl. Anim. Behav. Sci. 120:28-38.

Petherick, J. C., R. G. Holroyd, V. J. Doogan, and B. K. Venus. 2002. Productivity, carcass and meat quality of lot-fed *Bos indicus* cross steers grouped according to temperament. Aust. J. Exp. Agr. 42:389-398.

Petherick, J. C., R. G. Holroyd, and A. J. Swain. 2003. Performance of lot-fed *Bos indicus* steers exposed to aspects of a feedlot environment before lot-feeding. Aust. J. Exp. Agr. 43:1181-1191.

Pfiffener, J.J. 1942. The adrenal cortical hormones. Advan. Enzymol. 2:325-356.

Pohorecky, L. A. and R. J. Wurtman. 1971. Adrenocortical control of epinephrine synthesis. Pharmacol. Rev. 23:1-35.

Pounden, W. D., and J. G. Firebaugh. 1956. Effects of nervousness on conception during artificial insemination. Vet. Med. 51:469-470.

Purcell, D., C. W. Arave, and J. L. Walters. 1988. Relationship of three measures of behavior to milk production. Appl. Anim. Behav. Sci. 21:307-313.

Rabadan-Diehl C., S. J. Lolait, and G. Aguilera. 1995. Regulation of pituitary vassopressin V1b receptor mRNA during stress in the rat. J. Neuroendocrinol. 7:903-910.

Reul, J. M., and E. R. de Kloet. 1985. Two receptor systems for corticosterone in rat brain: microdistribution and differential occupation. Endocrinol. 117:2505-2511.

Riniker, B., P. Sieber, and W. Rittel. 1972. Revised amino-acid sequences for porcine and human adrenocorticotrophic hormone. Nature New Biol. 253:114-115.

Rivier, C., and W. Vale. 1987. Diminished responsiveness of the hypothalamic–pituitary–adrenal axis of the rat during exposure to prolonged stress: a pituitary-mediated mechanism. Endocrinol. 121:1320-1328.

Rolfe, K. M., W. M. Snelling, M. K. Nielsen, H. C. Freetly, C. L. Ferrell, and T. G. Jenkins. 2011. Genetic and phenotypic parameter estimates for feed intake and other traits in growing beef cattle, and opportunities for selection. J. Anim. Sci. 89:3452-3459.

Royce, P. C. and G. Sayers. 1960. Purification of hypothalamic corticotropin releasing factor. Proc. Soc. Exptl. Biol. Med. 103:447-450.

Salomé, N., O. Viltart, J. Lesage, R. Landgraf, D. Vieau, and C. Laborie. 2006. Altered hypothalamo–pituitary–adrenal and sympatho-adrenomedullary activities in rats bred for high anxiety: central and peripheral correlates. Psychoneuroendocrinol. 31:724–735.

Sapolsky, R. M., L. M. Romero, and A. U. Munck. 2000. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. Endocr. Rev. 21:55-89.

Satterlee, D. G., and W. A. Johnson. 1988. Selection of Japanese quail for contrasting blood corticosterone response to immobilization. Poult. Sci. 67:25–32.

Scott, J. P., and E. Fredericson. 1951. The causes of fighting in mice and rats. Physiol. Zool. 24:273-309.

Selye, H. 1936. A syndrome produced by diverse nocuous agents. Nature 138:32.

Selye, H. 1956. The Stress of Life. Revised edition. McGraw-Hill, New York.

Selye, H. 1971. Hormones and resistance. J. Pharm. Sci. 60:1-28.

Selye, H. 1973. The evolution of the stress concept. Am. Sci. 61: 692-699.

Senn, M., P. M. Maier, and W. Langhans. 1995. ACTH, cortisol and glucose responses after administration of vasopressin in cattle and sheep. J. Comp. Physiol. [B] 164:570-578.

Smith, P. E. 1930. Hypophsectomy and a replacement therapy in the rat. Am. J. Anat. 45:205-273.

Stachenko, J., and C. J. P. Giroud. 1959. Functional zonation of the adrenal cortex: site of ACTH action. Endocrinol. 64:743-752.

Steimer, T., and P. Driscoll. 2005. Inter-individual vs line/strain differences in psychogenetically selected Roman high-(RHA) and low-(RLA) avoidance rats: neuroendocrine and behavioural aspects. Neurosci. Biobehav. Rev. 29:99–112.

Steimer, T., A. Python, P. E. Schulz, and J. M. Aubry. 2007. Plasma corticosterone, dexamethasone (DEX) suppression and DEX/CRH tests in a rat model of genetic vulnerability to depression. Psychoneuroendocrinol. 32:575-579.

Stenzel-Poore, M., W. W. Vale, and C. Rivier. 1993. Relationship between antigen-induced immune stimulation and activation of the hypothalamic-pituitary-adrenal axis in the rat. Endocrinol. 132:1313-1318.

Stricklin, W. R., and C. C. Kautzscanavy. 1984. The role of behavior in cattle production - A review of research. Appl. Anim. Ethol. 11:359-390.

Sydnor, K.L., and G. Sayers. 1954. Blood and pituitary ACTH in intact and adrenalectomized rats after stress. Endocrinol. 55:621-636.

Trapp, T., R. Rupprecht, M. Castren, J. M. Reul, and F. Holsboer. 1994. Heterodimerization between mineralocorticoid and glucocorticoid receptor: a new principle of glucocorticoid action in the CNS. Neuron 13:1457–1462.

Toutain, P. L., R. A. Brandon, M. Alvinerie, R. Garcia-Villar, and Y. Ruckebusch. 1982. Dexamethasone in cattle: pharmacokinetics and action on the adrenal gland. J. Vet. Pharmacol. Therap. 5:33-43.

Tsuang, M. T., S. V. Faraone, and M. J. Lyons. 1993. Identification of the phenotype in psychiatric genetics. Eur. Arch. Psychiat. Clin. Neurosci. 243:131–142.

Tulloh, N. M. 1961. Behaviour of cattle in yards. II. A study in temperament. Anim. Behav. 9:25-30.

Vale, W., J. Spiess, C. Rivier, and J. Rivier. 1981. Characterization of a 41-residue ovine hypothalamic peptide that stimulates secretion of corticotropin and beta-endorphin. Science 213:1394-1397.

Van Reenen, C. G., N. E. O'Connell, J. T. N. Van der Werf, S. M. Korte, H. Hopster, R. B. Jones, and H. J. Blokhuis. 2005. Responses of calves to acute stress: Individual consistency and relations between behavioral and physiological measures. Physiol. Behav. 85:557-570.

Veissier, I., C. G. Van Reenen, S. Andanson, and I. E. Leushuis. 1999. Adrenocortictropic hormone and cortisol in calves after corticotropin-releasing hormone. J. Anim. Sci. 77:2047-2053.

Voisinet, B. D., T. Grandin, S. F. OConnor, J. D. Tatum, and M. J. Deesing. 1997a. *Bos indicus* cross feedlot cattle with excitable temperaments have tougher meat and a higher incidence of borderline dark cutters. Meat Sci. 46:367-377.

Voisinet, B. D., T. Grandin, J. D. Tatum, S. F. OConnor, and J. J. Struthers. 1997b. Feedlot cattle with calm temperaments have higher average daily gains than cattle with excitable temperaments. J. Anim. Sci. 75:892-896.

von Bardeleben, U., and F. Holsboer. 1989. Cortisol response to a combined dexamethasone-hCRH challenge in patients with depression. J. Neuroendocrinol. 1:485–488

Wagner, W. C., and S. L. Oxenrider. 1972. Adrenal function in the cow. Diurnal changes and the effects of lactation and neurohypophyseal hormones. J. Anim. Sci. 34:630-635.

Walker, C. D., R. W. Rivest, M. J. Meaney, M. L. Aubert. 1989. Differential activation of the pituitary–adreno–cortical axis after stress in the rat:use of two genetically selected lines (Roman high- and low-avoidance rats). J. Endocrinol. 123:477-485.

Wigger, A., M. M. Sánchez, K. C. Mathys, K. Ebner, D. Liu, A. Kresse, I. D. Neumann, F. Holsboer, P. M. Plotsky, and R. Landgraf. 2004. Alterations in central neuropeptide expression, release, and receptor binding in rats bred for high anxiety: critical role of vasopressin. Neuropsychopharmacol. 29:1-14.

Windle, R. J., S. A. Wood, S. L. Lightman, and C. D. Ingram. 1998. The pulsatile characteristics of hypothalamo-pituitary-adrenal activity in female Lewis and Fischer 344 rats and its relationship to differential stress responses. Endocrinology. 139:4044-4052.

Young, E. A., J. Abelson, and S. L. Lightman. 2004. Cortisol pulsatility and its role in stress regulation and health. Front. Neuroendocrinol. 25:69–76.

Zavy, M. T., P. E. Juniewicz, W. A. Phillips, and D. L. VonTunglen. 1992. Effect of initial restraint, weaning, and transport stress on baseline and ACTH-stimulated cortisol responses in beef calves of different genotypes. Am. J. Vet. Res. 53:551-557.

VITA

Name:	Kevin Owen Curley Jr.
Address:	Department of Veterinary Integrative Biosciences Texas A&M University College Station, TX 77843-4458
Email Address:	kcurley@cvm.tamu.edu
Education:	B.S., Animal Science, University of Rhode Island, 2001 M.S., Physiology of Reproduction, Texas A&M University, 2004